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Chemical Composition, Insecticidal Activity of Essential Oil and Powder of Cyperus Rotundus L. 1753 Against Callosobruchus Maculatus

Abdoulaye Thiam, Momar Talla Gueye, Serigne Mbacké Diop, Papa Seyni Cissokho and El Hadji Barka Ndiaye

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Chemical composition, insecticidal activity of essential oil and powder of *Cyperus rotundus* L. 1753 against *Callosobruchus maculatus*

Abdoulaye Thiam [1,2*[0000-0001-8499-1064]], Momar Talla Guèye [2[0000-0002-7459-1080]], Serigne Mbacké Diop [1,2[0000-0001-6903-9438]], Papa Seyni Cissokho [2], and Elhadji Barka Ndiaye [3[0000-0002-7895-4299]]

[1] Faculty of Science and Technology, Department of Chemistry, Cheikh Anta Diop University, Dakar, Senegal

[2] Laboratoire des Analyses Phytosanitaires, Institut de Technologie Alimentaire, Dakar, Senegal

[3] Faculty of Fundamental and Engineering Sciences, Sine Saloum El hadji Ibrahima Niass University, Kaolack, Senegal

*Corresponding address: athiam747@yahoo.fr

Abstract. Synthetic pesticides used in the preservation of cereals and legumes cause enormous damage to human health and the environment. To gradually replace them, the use of natural products is the best alternative. The aim of this study is to evaluate the toxicity of the essential oil and powder of *Cyperus rotundus* against *Callosobruchus maculatus*, the main cowpea pest in Senegal. For this purpose, the essential oil was extracted by steam distillation while the powder was obtained by grinding the rhizomes. The chemical characterization of the essential oil and the powder was done by GC-MS/FID and GC-MS/HS-SPME respectively. The insecticidal activity was evaluated by fumigation for the essential oil and by contact for the powder. The main compounds identified in the essential oil were humulene oxide II (26.1±1.3%), caryophyllene oxide (19.2±0.7%) and longiverbenone (11.3±0.6%), while in the powder caryophyllene oxide (18.7±2.8%), α-ylangene (14.1±0.4%) and α-cubebene (11.2±0.9%) were in the majority. This qualitative and quantitative difference noted on the two chemical profiles would be due to the method and duration of extraction of the volatile constituents. Both the essential oil and the powder of *C. rotundus* showed efficacy against *C. maculatus*. For the essential oil an LD_{50} of 0.19 µl.cm^{-3} and a LT_{50} of 63.9 hours were recorded. As for the powder, the LD_{50} and LT_{50} obtained are respectively 27.0 mg.g^{-1} and 65.3 hours. The powder was more effective against *C. maculatus* in the long term than the essential oil. For a better valorization of the results, it is preferable to use the powder, which is more accessible to rural populations. As for the oil, it could be fixed on a support to facilitate its use. Moreover, a bio-insecticide based on essential oil and clay is currently being formulated.

Keywords: *Cyperus rotundus*, Essential oil, Plant powder, *Callosobruchus maculatus*, Bio-insecticide

1 Introduction

In sub-Saharan Africa, and particularly in Senegal, the diet of the population is essentially based on cereals and legumes. Indeed, cowpea (*Vigna unguiculata* L. Walp) is, after groundnut, the most important staple legume. However, during the storage period, severe attacks by *Callosobruchus maculatus* are often recorded. Despite all the remarkable efforts made to increase this crop production, post-harvest losses, usually caused by insect pests, are often noted during storage [1]. In this regard, if no protection is applied, losses of stored foodstuffs can reach 100% [2-4]. However, to cope with insect depredation, synthetic pesticides are generally used in post-harvest [5]. Notwithstanding their effectiveness, their use is limited or even prohibited in some countries due to recorded cases of poisoning. In addition, their progressive use generally leads to environmental nuisance and the development of a certain parasitic resistance, in particular *Tribolium castanum* and *Callosobruchus maculatus* which have developed resistance to lindane and phosphine respectively [6]. Furthermore, in recent years, due to the renewed interest in natural products, essential oils and plant powders are widely used to address this threat to human health and also as an alternative in the control of crop pests. It should be noted that Senegal has a rich flora of aromatic plants but very few studies. The objective of this study is to valorize the Senegalese flora. Firstly, we will characterize the essential oil and the powder, respectively by GC-MS/FID (gas chromatography coupled with mass spectrometry and a flame ionization detector) and GC/MS-HS-SPME (gas chromatography and mass spectrometry by solid phase micro extraction). Secondly, to test the insecticidal potential of the essential oil and plant powder of *C. rotundus* against *C. maculatus*, the main cowpea pest in Senegal.

2 Materials and methods

2.1 Materials

Rearing of the cowpea bruchid, *Callosobruchus maculatus*

The insects were reared at the Laboratoire des Analyses Phytosanitaires at the Institut de Technologie Alimentaire in jars containing cowpeas (variety "Yacine"). They were recovered after sieving the cowpea grains. These

individuals were isolated and placed in other jars until the adults of the next generation appeared at the ambient temperature of the laboratory (28 to 30°C). This last generation was used to test the insecticidal activity of the essential oil and the powder of *C. rotundus* by contact and fumigation respectively.

Cowpea (*Vigna unguiculata*)

Cowpea seeds were purchased at a local market in Dakar, Senegal. To avoid insect infestation, 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 being used. Seeds showing any damage were discarded.

Harvesting *Cyperus rotundus*

Rhizomes of *C. rotundus* were collected in June 2018 in the village of Mlomp located between 12° 33' 11" north latitude and 16° 35' 39" west longitude in Lower Casamance, about 40 km southwest of Ziguinchor. A specimen has been deposited in the herbarium of the " Institut Fondamental d'Afrique Noire (IFAN) " of Cheikh Anta DIOP University, Dakar.

2.2 Methods

Chromatographic analysis of *Cyperus rotundus* essential oil by GC/FID

A GC Trace Ultra (Thermo Electron Corporation, Interscience, Milan, Italy) coupled to a flame ionization detector was used. The operation of the oven in GC/FID is the same as in GC/MS. It was first maintained in isothermal mode at 40 °C for 5 minutes. Then the temperature was gradually increased by 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 rate of 30 ml.min^{-1}. The detector temperature was 290 °C. Helium was the carrier gas with a constant flow rate of 1.5 ml.min^{-1}. The capillary column used was Optima-5-accent (HP-5MS) 5% phenylmethylsiloxane (Macherey-Nagel, Germany). It has a length of 30 m, a diameter of 0.25 mm and a film thickness of 0.25 µm. The volume of the injected sample was 1 µl (10 mg/10 ml n-hexane). The air and hydrogen flow rates were 350 and 35 ml.min^{-1}, respectively.

Chromatographic analysis of Cyperus rotundus essential oil by GC/MS

The Agilent technologies 5973 Network Mass Selective Detector quadrupole mass spectrometer was combined with a gas chromatograph, Agilent technologies 6890N (G1530N), USA. The relative abundance of the spectral peaks ranged from 50 to 550 m/z, at an ionisation energy of 70 eV. The identification of the compounds in the essential oils of *C. rotundus* was carried out by comparing the mass spectra obtained with those in the computerised database and the retention indices with those given in the literature [7, 8]. The percentage of each constituent of the essential oil was calculated by the area normalization method from the GC peak areas calculated as the average value of two injections of each oil. The identification of the compounds in the chromatographic profiles was performed by comparing their mass spectra with a library database. This was confirmed by comparing the retention indices calculated using a series of C7-C30 n-alkanes injected under the same conditions as the essential oil with those of authentic standards (α-copaene, δ-selinene, caryophyllene oxide, humulene II oxide, longiverbenone) or with literature values.

Chromatographic analysis of *Cyperus rotundus* powder by GC/MS-HS-SPME

The type of SPME fibre used was PDMS/CAR/DVB. Its core is designed from fused silica. With a film thickness ranging from 50 to 30 µm, the SPME fibre resembled a retractable syringe at the end. It had a 23 gauge needle (OD 0.64 mm) and a length of 10 mm. Deug (2) g of substrate (powder) was used for the analysis. First, the oven was held at 50°C for 1 minute. Then, the temperature gradually was increased from 4 °C.min-1 to 200 °C for 5 min, and 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. The HS-SPME required the injector to be operated in minus split mode to allow full desorption of the volatile organic compounds in the Optima-5-accent, 5%-phenyl-95%-methylsiloxane capillary column (Macherey-Nagel, Düren-Germany). It has a length of 30 m, a diameter of 0.25 mm and a film thickness of 0.25 µm. Desorption took 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 an Agilent technologies 7890A. It was associated with a gas chromatograph. The relative abundance of the spectral peaks was between 40 and 400 m/z, for an ionisation energy of 70 eV. The identification of the molecules was the same as for the GC/MS analysis.

Insecticidal activity of the essential oil of *Cyperus rotundus*

The insecticidal activity of the essential oil of *C. rotundus* was evaluated by fumigation. The experimental protocol was inspired by the work of Kouninki et al [9] with some modifications. Thus, ten (10) adult insects were

introduced into Petri dishes of 9 cm diameter, the upper part of each of which was glued with a cotton ribbon of about 3 mm thickness, 1 cm width and 3 cm length. Then, 10 g of healthy cowpea seeds used as substrate was introduced into each Petri dish. Then, three (3) concentrations of *C. rotundus* essential oil were prepared separately in 50 µL of Acetone (Ac) (HE µl : Ac µl) in the following ratios 12.5:50.0; 25.0:50.0 and 50.0:50.0, noted T2, T3 and T4 respectively. A blank and an acetone control, labelled T0 and T1 respectively, were also used (Table 1). The tests were repeated 3 times for each concentration. Mortality was assessed 24, 48 and 72 hours after the first day of testing.

Table 1. Insecticidal test of *Cyperus rotundus* essential oil by fumigation

Treatments	**T0**	**T1**	**T2**	**T3**	**T4**
Doses (µL)	Blank	Acetone	12.5	25.0	50.0

Insecticidal activity of *Cyperus rotundus* powder

The insecticidal activity of the powder was evaluated by contact. The experimental protocol used was inspired by the work of Goléti [10]. The tests consisted of placing 100 g of cowpea seeds and *C. rotundus* powder in one-litre jars at doses of 2.0, 3.0 and 4.0 g, to be noted as treatments T2, T3 and T4 respectively. A blank control and an actellic control noted respectively T0 and T1 were also used (Table 2). In order to ensure a homogeneous distribution of the powder on the seeds, the jars were shaken manually for 2 to 3 minutes. Then, 20 unsexed adult C. maculatus insects, not older than two (2) days, were introduced into each jar. Each treatment was repeated 3 times.

Table 2. Insecticidal test of *Cyperus rotundus* powder by contact

Treatments	**T0**	**T1**	**T2**	**T3**	**T4**
Doses (g)	Blank	Actellic	2.0	3.0	4.0

Mortality was assessed for 7 days after the first day of testing. Insects that died during the treatment were counted and removed and the survivors returned to their original jars. The Abbott formula [11] below was used to calculate the mortality of insects in both the fumigation and contact treatments.

$$\% \mathbf{M} = \left(\frac{\mathrm{Mx} - \mathrm{Mt}}{100 - \mathrm{Mt}}\right) \mathrm{x}100$$

Where : % M = Mortality; Mx = Mortality recorded in sample x and Mt = Mortality in the blank control

3 Statistical analysis

The statistical analysis was performed with XLSTAT-Pro 6.1.9 software and the treatment data were analysed and compared by analysis of variance (ANOVA). The extraction results of essential oil yield and insecticidal activity were expressed as mean values ± standard deviation (n = 3), and differences with $p < 0.05$ were considered significant according to the Tukey test.

4 Results and discussion

4.1 Results

Yield of essential oil extraction

Extraction of the essential oil by steam stripping of the root part of *C. rotundus* gave a yield of 0.4±0.1%.

Chemical composition of the essential oil of *Cyperus rotundus*

Twenty-five (25) compounds were identified in the essential oil, i.e. a total of 92.6±0.6% compounds identified (Table 3). It should be noted that the chemical composition of the essential oil of *C. rotundus* from Senegal is mainly dominated by sesquiterpenes. Indeed, oxygenated sesquiterpenes represent 70.6±0.9% of the total chemical composition. The main compounds identified are respectively humulene oxide II (26.1±1.3%), caryophyllene oxide (19.2±0.7%) and longiverbenone (11.3±0.6%). These three compounds represent 56.6% of the total chemical composition of the essential oil of *C. rotundus*. As for hydrocarbon sesquiterpenes, they constitute 14.4±0.8% of

the total content of identified compounds and are dominated by α-copaene (3.5±0.2%), α-cubebene (3.7±0.2%), δ-Selinene (2.0±0.1%). For monoterpenes, oxygenated monoterpenes represent 7.5±0.1% of the total chemical composition and among them are trans-pinocarveol (1.8±0.1%), myrthenal (1.7±0.0%) and verbenone (1.2±0.1%) It is worth mentioning the complete absence of hydrocarbon monoterpenes in the essential oil of *C. rotundus*.

Table 3. Chemical composition of *Cyperus rotundus* essential oil

Compounds	**RT (min.)**	**RI**	**Composition (%)**	**Identification methods**
trans-Pinocarveol	10.375	1152	1.8±0.1	MS, RI
Myrthenal	11.773	1194	1.7±0.0	MS, RI
Verbenone	12.115	1203	1.2±0.1	MS, RI
α-Copaene	16.349	1340	3.5±0.2	MS, RI, Std
α-Cubebene	16.472	1346	3.7±0.2	MS, RI
δ-Selinene	17.152	1374	2.0±0.1	MS, RI, Std
9,10-Dihydroisologifolene	17.3	1381	0.8±0.0	MS, RI
uc	18.777	1501	0.7±0.1	-
α-Guaiene	18.896	1504	0.5±0.0	MS, RI
γ-Cadinene	19.276	1516	1.4±0.0	MS, RI
β-Chamigrene	19.504	1523	0.6±0.0	MS, RI
Valencene	19.744	1530	1.0±0.1	MS, RI
uc	20.314	1548	0.4±0.1	-
uc	20.572	1556	0.9±0.0	-
Calacorene	21.133	1573	0.6±0.0	MS, RI
cis-Caryophyllene oxide	21.395	1581	1.5±0.0	MS, RI
Caryophyllene oxide	22.252	1608	19.2±0.7	MS, RI, Std
Humulene oxide II	22.974	1632	26.1±1.3	MS, RI, Std
Viridiflorol	23.502	1649	1.2±0.2	MS, RI
(-)-Caryophyllene oxide	23.612	1653	1.6±0.8	MS, RI
4,4-Dimethyltetracyclo-[6,3,2,0]tridecan-9-ol	23.717	1656	0.5±1.8	MS, RI
Nerolidol	23.971	1664	0.5±0.3	MS, RI
Isoaromadendrene epoxide	24.363	1677	3.7±0.3	MS, RI
uc	24.743	1689	2.1±0.6	-
Longiverbenone	24.84	1693	11.3±0.6	MS, RI, Std
(+)(-)-Caryophyllene oxide	24.954	1696	1.9±0.3	MS, RI
Aristolone	25.288	1707	1.4±0.2	MS, RI
uc	25.529	1716	0.6±0.1	MS, RI
uc	26.09	1735	3.4±0.1	-
Limonene dioxide	27.28	1776	2.8±0.1	MS, RI
Nootkatone	28.172	1807	1.3±0.0	MS, RI
Hydrocarbon Monoterpenes			**0.0±0.0**	
Oxygenated monoterpenes			**7.5±0.1**	
Hydrocarbon sesquiterpenes			**14.4±0.8**	
Oxygenated sesquiterpenes			**70.6±0.9**	
Unidentified compounds (uc)			**7.4±0.6**	
Total compounds identified			**92.6±0.6**	

MS= Mass Spectroscopy, RI= Retention Indices; Std= Standard

Chemical composition of *Cyperus rotundus* powder

Fifty (50) volatile compounds were identified in *C. rotundus* powder (Table 4). These are mainly caryophyllene oyde (18.7±2.8%), α-ylangene (14.1±0.4%), α-cubebene (11.2±0.9%), β-pinene (5.6±0.7%), α-piene (5.4±0.8%) and δ-selinene (4.6±0.1%). These majority compounds represent about 59.6% of the total chemical composition of the powder. All other compounds identified in the powder were identified at very low percentages. Among them we can mention 1,8-cineole (0.5±0.1%), trans-D-dihydrocarveol (0.4±0.0%), 4-terpineol (0.3±0.0%), carveol (0.2±0.0%) and camphene (0.1±0.0%). On the other hand, the total content of sesquiterpene hydrocarbon derivatives (48.8±0.7%) is twice as high as the oxygenated sesquiterpene derivatives (20.9±3.0%). In addition, the content of hydrocarbon monoterpenes (17.5±1.9%) is slightly higher than the content of oxygenated monoterpenes (11.5±0.7%).

Table 4. Chemical composition of *Cyperus rotundus* powder

Compounds	RT (min.)	RI	Composition (%)	Identification methods
α-Pinene	6.514	926	5.4±0.8	MS, RI, Std
Camphene	6.856	938	0.1±0.0	MS, RI
Verbenene	7.018	944	0.5±0.1	MS, RI
β-Pinene	7.753	969	5.6±0.7	MS, RI, Std
cni	8.529	996	0.2±0.0	-
p-Cymene	9.188	1016	1.9±0.2	MS, RI, Std
D-Limonene	9.359	1022	3.1±0.2	MS, RI, Std
1,8-Cineole	9.402	1023	0.5±0.1	MS, RI
trans-D-dihydrocarveol	11.296	1080	0.4±0.0	MS, RI
6-Camphenol	12.542	1117	0.1±0.0	MS, RI
trans-Pinane	12.911	1128	0.5±0.1	MS, RI
trans-Pinocarveol	13.041	1138	2.3±0.1	MS, RI
Verbenol	13.226	1156	0.6±0.1	MS, RI
Pinocarvone	13.824	1166	1.3±0.2	MS, RI, Std
2,6,6-trimethyl-bicyclo [3.1.1] heptan-3-one	14.177	1170	0.1±0.0	MS, RI
4-Terpineol	14.317	1181	0.3±0.0	MS, RI
p-Cymen-8-ol	14.652	1186	0.2±0.0	MS, RI
α-Terpineol	14.83	1188	0.4±0.0	MS, RI
Mytenal	15.003	1203	2.7±0.2	MS, RI
(1S) 4,6,6-trimethyl-bicyclo [3.1.1] hept-3-en-2-one	15.48	1215	2.2±0.1	MS, RI
Carveol	15.745	1249	0.2±0.0	MS, RI
Carvone	16.566	1449	0.2±0.0	MS, RI
1,2-dimethyl-3,5-bis(1-methylethenyl) cyclohexane	19.092	1410	0.3±0.0	MS, RI
cni	19.546	1424	0.1±0.0	-
3,9-Dodecadiyne	19.689	1428	0.1±0.0	MS, RI
α-Copaene	20.1	1441	0.7±0.0	MS, RI
Alloaromadendrene oxide-1	20.41	1451	0.2±0.0	MS, RI
α-Amorphene	20.673	1459	1.1±0.1	MS, RI
α-Ylangene	20.99	1469	14.1±0.4	MS, RI, Std
α-Cubebene	21.161	1474	11.2±0.9	MS, RI, Std
Sativene	21.446	1483	0.4±0.0	MS, RI

Compounds	RT (min.)	RI	Composition (%)	Identification methods
β-Elemene	21.535	1486	0.5±0.1	MS, RI
δ-Selinene	21.788	1493	4.6±0.1	MS, RI
Dihydroaromadendrene	21.939	1498	1.9±0.0	MS, RI
Caryophyllene	22.334	1511	0.6±0.1	MS, RI
α-Humulene	23.328	1543	1.2±0.2	MS, RI
β-Selinene	23.579	1551	1.8±0.1	MS, RI
α-Guaiene	23.686	1555	1.3±0.0	MS, RI
α-Gurjunene	24.026	1566	0.3±0.0	MS, RI
γ-Cadinene	24.143	1570	2.6±0.1	MS, RI
α-Amorphene	24.364	1577	1.6±0.1	MS, RI
β-Chamigrene	24.631	1586	2.0±0.1	MS, RI
Valencene	24.807	1591	0.3±0.0	MS, RI
α-Muurolene	25.188	1604	0.1±0.0	MS, RI
8-Cedren-13-ol	25.281	1607	0.2±0.0	MS, RI
Eremophilene	25.498	1615	1.0±0.0	MS, RI
δ-Cadinene	26.057	1634	0.3±0.0	MS, RI
α-Calacorene	26.347	1644	0.9±0.1	MS, RI
Caryophyllene oxide	28.25	1709	18.7±2.8	MS, RI, Std
Longiverbenone	29.928	1770	0.9±0.1	MS, RI
Nootkatone	30.09	1776	0.6±0.1	MS, RI
cni	31.19	1816	0.8±0.1	-
2-Methylenecyclododecanone	31.85	1841	0.5±0.0	MS, RI
Hydrocarbon Monoterpenes			**17.5±1.9**	
Oxygenated monoterpenes			**11.5±0.7**	
Hydrocarbon sesquiterpenes			**48.8±0.7**	
Oxygenated sesquiterpenes			**20.9±3.0**	
Unidentified compounds (uc)			**1.1±0.1**	
Total compounds identified			**98.9±0.2**	

MS= Mass Spectroscopy, RI= Retention Indices; Std= Standard

Insecticidal activity of *Cyperus rotundus* essential oil against *Callosobruchus maculatus*

Figure 1 below presents the efficacy of *C. rotundus* essential oil by fumigation on C. *maculatus* mortality. It shows that treatments T2, T3 and T4 are effective after 24, 48 and 72 hours compared to treatments T0 (white control) and T1 (acetone). For the latter, no mortality was recorded. Treatments T2, T3 and T4 with respective mortalities of 6.7, 10.0 and 16.7% showed no significant difference after 24 hours of treatment. After 48 hours of treatment, T4 caused significantly more mortality of *C. maculatus*, i.e. 40.0%, than T2 (13.3%). The same observation is noted after 72 h of fumigation where T2 gives 36.7% mortality against 66.7% for T4 with an LD_{50}= 0.19 µl.cm^{-3}. It should be noted that treatment T3 with 60.0% mortality is statistically equal to treatment T4, with lethal times (LT_{50}) 63.9 and 56.5 hours respectively. In sum, the insecticidal effect of fumigation with *C. rotundus* essential oil against *C. maculatus* increases considerably with the increase of the dose used and the duration of exposure of the insects.

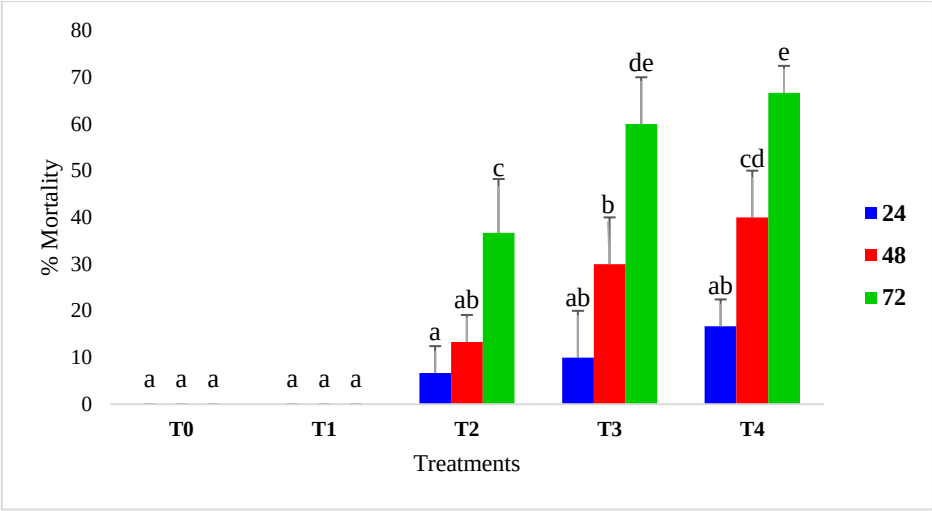

Fig. 1. Toxicity of *Cyperus rotundus* essential oil against *Callosobruchus maculatus*
For the same time, the mortality rates followed by the same letter are not significantly different at the 5% level.

Insecticidal activity of *Cyperus rotundus* powder against *Callosobruchus maculatus*

Figure 2 shows the mortality of *C. maculatus* as a function of the treatment with *C. rotundus* powder. Treatments T2, T3 and T4 showed a clearly significant difference from T0 from day 2 of evaluation, in contrast to treatment T1 (actellic) where 100.0% mortality was recorded. On the 3rd day of evaluation, the mortalities allowed the determination of the LD_{50} and LT_{50}, which correspond, to 39.0 mg/g and 75.2 hours respectively. Furthermore, on the 4th day of contact treatment with *C. rotundus* powder (LD_{50}=28.0 mg/g), 45.0, 46.7 and 61.7% mortality were recorded for treatments T2, T3 and T4 respectively. On day 5 of evaluation, mortality for T3 (58.3%) and T2 (60.0%) was relatively the same. On day 6 of treatment, 75.0, 76.7 and 100.0% mortality were recorded for T2, T3 and T4 respectively with LT_{50} of 102.7 and 97.2 hours for T2 and T3 respectively and an LD_{95}= 38.0 mg/g for treatment T4. Furthermore, treatments T2 and T4 showed no significant difference on days 1 and 7 of treatment.

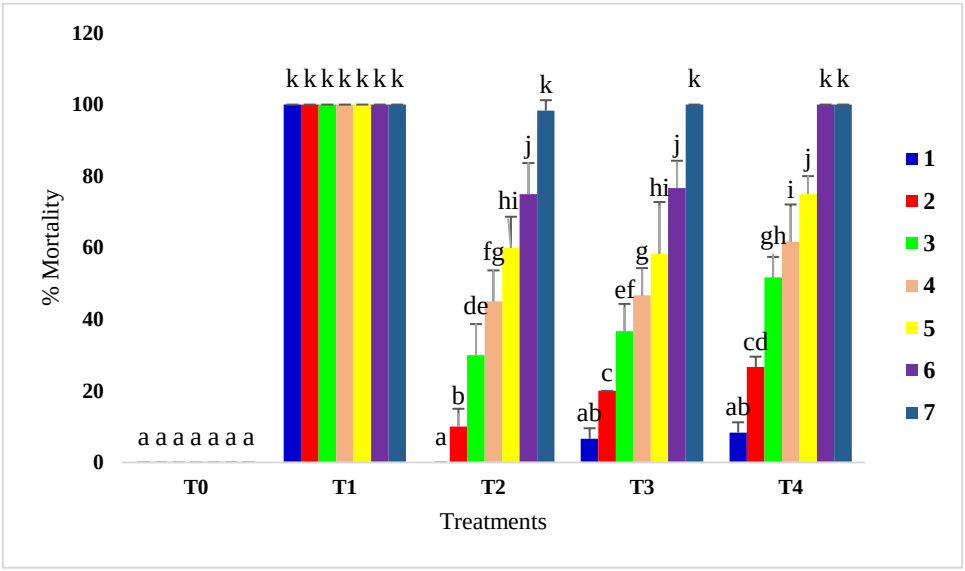

Fig. 2. Toxicity of *Cyperus rotundus* powder against *Callosobruchus maculatus*
For the same time, the mortality rates followed by the same letter are not significantly different at the 5% level.

5 Discussion

5.1 Yield

The extraction yield of essential oil from *C. rotundus* (0.4±0.1%) is similar to that obtained by Reham et al [12] in Egypt (0.4%). However, it is much lower than the yield given by Yagi et al [13] from Sudan, which is 2.6%, by the hydrodistillation method. This difference in yield depends on several factors including the place and time of

harvesting, the duration and method of extraction, the organ studied and the vegetative stage of the species [14-16].

Chemical composition of the essential oil of *Cyperus rotundus*

The study of the composition of the essential oil of *C. rotundus* from Senegal allowed the identification of the following compounds: humulene oxide II, caryophyllene oxide and longiverbenone. It should be noted that the studies carried out on the characterization of the essential oil of *C. rotundus* are numerous and varied [17-19]. Thus, depending on the locality, the chemical composition of the essential oil varies qualitatively and quantitatively. Indeed, the chemical composition of the essential oil of *C. rotundus* from Senegal is similar to that of the same species identified in Nigeria by Lawal al [20]. Furthermore, in the literature, some studies have shown qualitative and quantitative differences in the chemical composition of *C. rotundus* essential oil. Among them, we can mention the work of Yagi et al [13] in Sudan who obtained mainly 2,5,9-trimethylcycloundeca-4,8-dienone (13.44%), alloaromadendrene oxide-(1) (8.47%), piperitone (7.37%), β-lemol (7.14%), selina-6-en-4-ol (7%) and longiverbenone (4.30%). Of all the major compounds in the essential oil of *C. rotundus* from Sudan, only longiverbenone is present in the majority (11.3±0.6%) in the essential oil of *C. rotundus* from Senegal. On the other hand, the work of Lui et al [21] from China revealed a chemical composition of *C. rotundus* essential oil consisting of α-cyperone (29.38%), cyperene (13.97%), caryophyllene oxide (6.71%) and β-selinene (6.47%). It is noteworthy that in the chemical composition of the Senegalese essential oil, the content of caryophyllene oxide far exceeds that contained in the Chinese essential oil sample [21]. In South Africa, a study on the chemical characterization of C. *rotundus* essential oil from two localities revealed qualitatively and quantitatively different compositions. For the first locality, the chemical composition is dominated by α-cyperone (11.0%), myrtenol (7.9%), caryophyllene oxide (5.4%) and β-pinene (5.3%), while the second locality revealed β-pinene (11.3%), α-pinene (10.8%), α-cyperone (7.9%), myrtenol (7.1%) and α-selinene (6.6%) as main compounds [22]. Although the harvests were made in the same country but in different localities, the chemical composition of *C. rotundus* rhizomes remains different not only qualitatively but also quantitatively compared to our work on the same species. Moreover, this qualitative and quantitative difference in the chemical composition of *C. rotundus* essential oil has been reported in several studies on the same species [22-24].

5.3 Chemical composition of *Cyperus rotundus* powder

Volatile constituents of aromatic powders are often analysed by headspace solid phase microextraction coupled with detection by gas chromatography and HS-SPME/GC-MS [25-27]. However, in the literature, little work has been done on the characterization of *C. rotundus* powder by the HS-SPME method [18]. The particularity between the different HS-SPME characterizations lies in the nature of the fibre used. In this study, the characterization of *C. rotundus* powder was done using a PDMS/CAR/DVB fibre. This fibre was chosen because of its affinity with volatile and semi-volatile molecules. Indeed, this analysis allowed to identify mostly caryophyllene oxide, α-ylangene, α-cubebene, β-pinene, α-pinene and δ-selinene. The results are different from those obtained by Eröz Poyraz et al [28] in Turkey who identified predominantly, with a polydimethylsiloxane/dimethylbenzene (PDMS/DVB) fibre and for a 15-minute incubation at a temperature of 40 °C, cyperene (28.0%), α-copaene (12.0%), α-ylangene (10.5%) and rotundene (8.7%). These qualitative and quantitative differences recorded would be due to the nature of the fibre and the experimental parameters. In addition, the majority compounds represent 59.6% of the volatile compounds in *C. rotundus* powder. Thus, *C. rotundus* powder is dominated by hydrocarbon sesquiterpenes and oxygenated sesquiterpenes. This result is in perfect agreement with the work of Kilani et al [29], who obtained 70.0% of sesquiterpene derivatives. This abundance of sesquiterpenes in *C. rotundus* powder can be explained by their affinity with the PDMS/CAR/DVB fibre which gives a good extraction efficiency for a wide range of volatile and semi-volatile compounds. Indeed, this affinity can be attributed to the fact that larger molecules can be adsorbed by the DVB layer, while smaller molecules are attracted to the polydimethylsiloxane/carboxen (PDMS/CAR) coating [28]. Qualitatively, the chemical composition of *C. rotundus* powder from Senegal is comparable to that from China. However, the α-ylangene, which is among the majority compounds of the Senegalese *C. rotundus* species, was not identified in the same species in China [30]. The high presence of α-ylangene in *C. rotundus* powder is thought to be related to the concentration of the molecule in the sample, the extraction temperature, the absorption/desorption time and its affinity with the PDMS/CAR/DVB fibre [30]. It should be noted that *C. rotundus* powder generally consists of volatile molecules of low and medium polarity. Thus, the use of PDMS/CAR/DVB fibre favours more the extraction of hydrocarbon monoterpenes such as α-pinene, β-pinene and p-cymene, at the expense of some oxygenated monoterpenes such as mytenal, pinocarvone, verbenol and p-cymen-8-ol.

5.4 Insecticidal activity of the essential oil of *Cyperus rotundus* against *Callosobruchus maculatus*

Marked by the presence of three oxygenated monoterpenes: trans-pinocarveol, myrtenal and verbenone, the chemical composition of the essential oil of *C. rotundus* from Senegal consists mainly of sesquiterpene molecules.

Despite the low content of monoterpenes (4.7%), known for their insecticidal properties, the essential oil of *C. rotundus* was also toxic against *C. maculatus.* Evaluation of the insecticidal activity by fumigation of *C. rotundus* essential oil against *C. maculatus* resulted in 66.7% mortality with an LD50 of 0.19 µl.cm^{-3} after 72 hours. This insecticidal activity would probably be linked to the presence of the main compounds of this essential oil, notably caryophyllene oxide, humulene oxide II and longiverbenone. It should be noted that there are several mechanisms and modes of action of essential oils against insect pests. Several studies have reported the repellent and insecticidal power of essential oils with a high content of caryophyllene oxide and longiverbenone [31-33]. In addition, some studies attribute insecticidal activity not only to the majority compounds in an essential oil, but to the synergistic effect that the molecules may have with each other [34, 35]. Representing 4.0% of the total chemical composition, alcoholic derivatives such as trans-pinocarveol, viridiflorol and nerolidol, play a determining role in the toxicity of *C. rotundus* essential oil against *C. maculatus* as it has been shown that alcoholic derivatives can affect the cuticle of the insect's exoskeleton and thus facilitate the entry of other volatile compounds [36].

5.5 Insecticidal activity of *Cyperus rotundus* powder against *Callosobruchus maculatus*

C. rotundus powder at different treatments (2.0g; 3.0g and 4.0g) showed a significant insecticidal effect against adults of *C. maculatus*. From these results, it can be said that the efficacy of *C. rotundus* is not only proportional to the amount of powder used, but also to the duration of treatment. The results obtained are in agreement with those of Guèye et al [37] and Ka et al [38]. According to these authors, the longer the treatment time and the higher the dose used, the greater the mortality. The presence of significant amounts of caryophyllene oxide, α-ylangene and α-cubebene could be at the origin of the insecticidal activity of *C. rotundus* powder against adults of *C. maculatus*. In addition, the insecticidal activity of caryophyllene oxide against *Tribolium confusum* has also been reported by the recent work of Ainane et al [30]. However, it should be noted that the mode of action of the major compounds (caryophyllene oxide, α-ylangene and α-cubebene) with the weevil receptors is still unclear. It should be noted that volatile and semi-volatile molecules of essential oils are good candidates to interfere with insect physiology by different mechanisms [40]. They can also act on GABA chloride channels, thus causing overexcitation followed by spasm and death of the insect [41]. In addition, certain oxygenated monoterpenes such as 1,8-cineole, α-terpineol, and 4-terpineol, although present in relatively small quantities in *C. rotundus* powder, have a remarkable insecticidal power against *C. maculatus* [42]. Indeed, a high binding affinity of the α-terpineol molecule with the octopamine receptor has been demonstrated in the American cockroach [43]. In addition, 1,8-cineole, known to be a strong acetylcholinesterase inhibitor, acts by blocking the octopamine receptor pathway in protein models and by affecting the insect GABA system [44, 45]. In addition, nootkatone, although at a low concentration in *C. rotundus* powder (0.6±0.1%), has already shown promising AchE inhibition activity [46]. Nootkatone has been shown to have repellent activity against *Sitophilus zeamais* and S*itophilus oryzae* with percentages of 93.1±2.3% and 67.2±6.8% respectively [47]. This compound could have exactly the same effect against *C. maculatus*. The insecticidal activity of *C. rotundus* could also be attributed to the abrasive effect of the powder on the cuticles of adult insects. In addition, *C. rotundus* powder would mainly have a reproductive inhibitory activity thanks to α-terpineol and p-cymene, which would respectively prevent egg laying (oviposition), and larval penetration (ovicide and/or larvicide) in the seed [48]. Thus, after testing (over 9 months), no emergence was observed on the substrate. In this respect, *C. rotundus* powder can be considered as a bioactive substance against adults and larvae of *C. maculatus*.

6 Conclusion

This study allowed us to determine the chemical profile of the essential oil and the powder of *C. rotundus*. Indeed, humulene oxide II, caryophyllene oxide and longiverbenone were in the majority in the essential oil whereas in the powder caryophyllene oxide, α-ylangene and α-cubebene were the main compounds. Compounds such as longiverbenone and caryophyllene oxide identified in the essential oil and powder are known to be insecticidal. It should be noted that both the essential oil and the powder of *C. rotundus* showed efficacy against *C. maculatus*, the main pest of cowpea in Senegal. However, the powder was more effective in the long term than the essential oil, which has a short-term insecticidal activity due to the volatility of the compounds. Thus, for a better valorization of the results, it is preferable to use the powder, which is more accessible to rural populations. As for the essential oil, it could be fixed on a support to facilitate its use.

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8 References

1. Guèye, M.T., Seck D., Wathelet, J.P., Lognay, G. : Lutte contre les ravageurs des stocks de céréales et de légumineuses au Sénégal et en Afrique occidentale : synthèse bibliographique. Biotechnol. Agron. Soc. Environ. 15 (1), 183-194 (2011).

2. Ngamo, L.S.T., Hance, T. : Diversité des ravageurs des denrées et méthodes alternatives de lutte en milieu tropical. Tropicultura. 25 (4), 215-220 (2007).

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

4. André, K.M., Michel, M.T.J., Mulamba, M., Alpha, S.M., Moïse, K.B.M. : Effet de la poudre de Tephrosia vogelii dans la conservation des graines de Niébé (*Vigna unguiculata* L. Walp.) en stock contre *Callosobruchus maculatus* F. à Mbujimayi (RD. Congo). 25(1) 3827-3835 (2015).

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

6. Ahmed, S., Khan, M.A., Ahmad, N.: Determination of Susceptibility Level of Phosphine in Various Strains of Dhora (*Callosobruchus maculatus* F.). 4 (3), 329-331 (2002).

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

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

9. Kouninki, H., Hance, T, Noudjou, F.A., Lognay, G., Malaise, F., Ngassoum, M.B., Mapongmetsem, P.M., Ngamo, L.S.T., Haubruuge, E.: Toxicity of some terpenoids of essential oils of *Xylopia aethiopica* from Cameroon against *Sitophilus zeamais* Motschulsky. J Appl Entomology. 131 (4), 269-274 (2007) doi: 10.1111/j.1439-0418.2007.01154.x

10. Nguemtchouin, M.M.G., Ngassoum, M.B., Ngamo, L.S.T., Gaudu, X., Cretin, M.: Insecticidal formulation based on *Xylopia aethiopica* essential oil and kaolinite clay for maize protection. Crop Protection. 29 (9), 985-991 (2010). doi: 10.1016/j.cropro.2010.06.007

11. Abbott, W.S.: A Method of Computing the Effectiveness of an Insecticide. Journal of Economic Entomology. 18 (2), 265-267 (1925) doi: 10.1093/jee/18.2.265a

12. Reham, M.S., Amal, F.S., Zaki, A.A., El-Gendy, A.N., Madiha, A.H., Zaghloul, A.M.: Chemical Composition, Antiviral and Cytotoxic Activities of Essential Oil from *Cyperus rotundus* Growing in Egypt: Evidence from Chemometrics Analysis. Journal of Essential Oil Bearing Plants. 648-659 (2020). doi: 10.1080/0972060X.2020.1823892

13. Datta, S., Dhar, S., Nayak, S.S., Dinda, S.C.: Hepatoprotective activity of *Cyperus articulatus* Linn.against paracetamol induced hepatotoxicity in rats. 5(1), 314-319 (2013).

14. Thiam, A., Guèye, M.T., Ndiaye, I., Diop, S.M., Ndiaye, E.B., Fauconnier, M.L., Lognay, G.: 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 (2018).

15. Diop, S.M., Diop, M.B., Guèye, M.T., Ndiaye, I., Ndiaye, E.H.B., Thiam, A., Fauconnier, M.L., Lognay, G.: Chemical Composition of Essential Oils and Floral Waters of *Ocimum basilicum* L. from Dakar and Kaolack Regions of Senegal. Journal of Essential Oil Bearing Plants. 21(2), 540-547 (2018). doi: 10.1080/0972060X.2018.1425640

16. Ndiaye, E.H.B., Diop, M.B., Gueye, M.T., Ndiaye, I., Diop, S.M., Fauconnier, M.L., Lognay, G.: Characterization of essential oils and hydrosols from senegalese *Eucalyptus camaldulensis* Dehnh. Journal of Essential Oil Research. 30(2), 131-141 (2018). doi: 10.1080/10412905.2017.1420554

17. Eltayeb, I.M., ELAmin, A.M., Elhassan I., Ayoub, M.H.: A Comparative Study of the Chemical Composition of Essential Oils of *Cyperus rotundus* L. (Cyperaceae) Growing in Sudan. American Journal of Research Communication. 4(9), 28-72 (2016).

18. He, M., Yan, P., Yang, Z.Y., Zhang, Z.M., Yang, T.B., Hong, L.: A modified multiscale peak alignment method combined with trilinear decomposition to study the volatile/heat-labile components in Ligusticum chuanxiong Hort - *Cyperus rotundus* rhizomes by HS-SPME-GC/MS. Journal of Chromatography. 1079, 41-50 (2018). doi: 10.1016/j.jchromb.2018.01.040

19. Umerie, S.: Physicochemical characterization and utilization of *Cyperus rotundus* starch. Bioresource Technology. 72(2), 193-196 (2000). doi: 10.1016/S0960-8524(99)00103-0.

20. Lawal, O.A., Ogunwando, I.A., Opuku, A.R., Ayedeji, A.O.: Chemical composition and antibacterial activity of essential oils from the rhizomes of *Cyperus papyrus* L. grown in South Africa. Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas. 15(3), 136-143 (2016).

21. Liu, X.C., Lu, X.N., Liu, Q.Z., Liu, Z.L.: Chemical Composition and Insecticidal Activity of the Essential Oil of *Cyperus rotundus* Rhizomes Against *Liposcelis bostrychophila* (Psocoptera: Liposcelididae). Journal of Essential Oil Bearing Plants. 19 (3), 640-647 (2016). doi: 10.1080/0972060X.2014.963168

22. Lawal, O.A., Oyedeji, A.: Chemical Composition of the Essential Oils of *Cyperus rotundus* L. from South Africa. Molecules. 14, 2909-2917 (2009).

23. Mojab, F., Vahidi, H., Nickavar, B., Kamali-Nejad, M.: Chemical components of essential oil and antimicrobial effects of rhizomes from *Cyperus rotundus* L. Journal of Medicinal Plants. 8 (32), 91-186 (2009).

24. Chen, Y., Zhao, Y., Wang, X., Liu, J., Huang, L., Peng, C.: GC-MS analysis and analgesic activity of essential oil from fresh rhizoma of *Cyperus rotundus.* Zhong Yao Cai. 34 (8), 1225-1229 (2011).

25. Xiao L., Lee, J., Zhang, G., Ebeler, S.E., Wickramasinghe, N., Seiber, J., Mitchell, A.E.: HS-SPME GC/MS characterization of volatiles in raw and dry-roasted almonds (Prunus dulcis). Food Chemistry. 151, 31-39 (2014). doi: 10.1016/j.foodchem.2013.11.052

26. Khairy, H.L., Saadoon, A.F., Zzaman, W., Yang, T.A., Mat-Easa A.: Identification of flavor compounds in rambutan seed fat and its mixture with cocoa butter determined by SPME-GCMS. Journal of King Saud University – Science. 30 (3), 316-323 (2018) doi: 10.1016/j.jksus.2017.03.001

27. Ning, L., Zheng, F.P., Chen, H.T., Lui, S.Y., Gu, C., Song, Z.G.: Identification of volatile components in Chinese Sinkiang fermented camel milk using SAFE, SDE, and HS-SPME-GC/MS. Food Chemistry. 129 (3), 1242-1252 (2011). doi: 10.1016/j.foodchem.2011.03.115

28. Eröz-Poyraz İ., Demirci, B., Küçük, S.: Volatiles of Turkish *Cyperus rotundus* L. Roots. Rec.Nat.Prod. 12 (3), 222-228 (2018). doi: 10.25135/rnp.26.17.06.044

29. Kilani. S., Ledauphin, J., Bouhel, I., Sghaier, M., B., Boubaker, J., Skandrine, I., Mosrati, R., Ghedira K., Barillier, D., Ghedira, L.C. : Comparative Study of *Cyperus rotundus* Essential Oil by a Modified GC/MS Analysis Method. Evaluation of Its Antioxidant, Cytotoxic, and Apoptotic Effects. Chemistry and Biodiversity. 5 (5) 729-742, (2008). doi: 10.1002/cbdv.200890069

30. Zhang, C., Qi, M., Shao, Q., Zhou, S., Fu, R.: Analysis of the volatile compounds in *Ligusticum chuanxiong* Hort. using HS-SPME–GC-MS. Journal of Pharmaceutical and Biomedical Analysis. 44 (2), 464-470 (2007). doi: 10.1016/j.jpba.2007.01.024

31. Khani; A., Heydarian, M.: Fumigant and repellent properties of sesquiterpene-rich essential oil from *Teucrium polium* subsp. *Capitatum* (L.). Asian Pacific Journal of Tropical Medicine. 7, 956-961 (2014). doi: 10.1016/S1995-7645(14)60169-3

32. K. M. Meepagala, G. Sturtz, C. C. Mischke, D. Wise, et S. O. Duke, « Molluscicidal activity of vulgarone B against ram's horn snail (*Planorbella trivolvis*). Pest. Manag. *Sci.* 60, 479-482 (2004). doi: 10.1002/ps.833

33. Joshi, R.C., Meepagala, K.M., Sturtz, G., Cagauan, A.G., Mendoza, C.O., Dayan, F.E., Duke, S.O.: Molluscicidal activity of vulgarone B from *Artemisia douglasiana* (Besser) against the invasive, alien, mollusc pest, *Pomacea canaliculata* (Lamarck). International Journal of Pest Management. 51, 175-180 (2005). doi: 10.1080/09670870500183161

34. Gallardo, A., Picollo M.I., González-Audino, P., Mougabure-Cueto, G.: Insecticidal Activity of Individual and Mixed Monoterpenoids of Geranium Essential Oil Against *Pediculus humanus* capitis (Phthiraptera: Pediculidae). Jnl. Med. Entom. 49, 332-335 (2012). doi: 10.1603/ME11142.

35. Miyazawa, M., Hideyuki, Tougo, Ishihara, M.: Inhibition of Acetylcholinesterase Activity by Essential Oil from *Citrus paradise*. Natural Product Letters. 15, 205-210 (2001) doi: 10.1080/10575630108041281

36. Cueto, G.M., Zerba, E., Picollo, M.I.: Biological effect of 1-dodecanol in teneral and post-teneral *Rhodnius prolixus* and *Triatoma infestans* (Hemiptera: Reduviidae). Mem. Inst. Oswaldo Cruz. 100, 59-61 (2005). doi: 10.1590/S0074-02762005000100012

37. Guèye, M.T., Cissokho, P.S., Goergen, G., Ndiaye, S., Seck, D., Guèye, G., Wathelet, J.P., Lognay, G.: Efficacy of powdered maize cobs against the maize weevil *Sitophilus zeamais* (Coleoptera: *Curculionidae*) in stored maize in Senegal. Int. J. Trop. Insect Sci. 32, 94-100 (2012) doi: 10.1017/S1742758412000148

38. Ka, A., Gueye, M.T., Diop, S.M., Cissokho, P.S., Gueye, A.N. : Etude de l'efficacité de la poudre et des cendres de balle de riz contre deux insectes ravageurs du riz stocké au Sénégal, *Sitophilus zeamais* (Motsch.) et *Tribolium castaneum* (Herbst). Int. J. Bio. Chem. Sci. 12, 1731 (2018). doi: 10.4314/ijbcs.v12i4.17

39. Ainane, A., Benhima, R., Khammour, F., Elkouali, M., Abba, E.H, Cherroud, S., Ainane, T. : Composition chimique et activité insecticide de cinq huiles essentielles. Proceedings Biosune. 1, 67-79 (2018).

40. Tampe, J., Parra, L., Huaiquil, K., Mutis, A., Quiroz, A.: Repellent Effect and Metabolite Volatile Profile of the Essential Oil of *Achillea millefolium* Against *Aegorhinus nodipennis* (Hope) (Coleoptera: Curculionidae). Neotrop Entomol. 44, 279-285 (2015) doi: 10.1007/s13744-015-0278-5

41. Bloomquist, J.R.: Chloride channels as tools for developing selective insecticides. Arch. Insect Biochem. Physiol. 54, 145-156 (2003). doi: 10.1002/arch.10112

42. Thiam, A., Guèye M.T., Sangharé, C.H. Cissokho P.S., Ndiaye, E.H.B., Diop, S.M., Diop, M.B., Fauconnier, M.L.: 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. Journal of Plant Protection Research. 61, 203-212 (2021) doi: 10.24425/JPPR.2021.137955

43. Enan, E.E.: Molecular and pharmacological analysis of an octopamine receptor from american cockroach and fruit fly in response to plant essential oils. Arch. Insect Biochem. Physiol. 59, 161-171 (2005). doi: 10.1002/arch.20076

44. Ratra, G.S., Kamita S.G., Casida, J.E.: Role of Human GABAA Receptor β3 Subunit in Insecticide Toxicity. Toxicology and Applied Pharmacology. 172, 233-240 (2001). doi: 10.1006/taap.2001.9154

45. Höld, K.M., Sirisoma, N.S., Ikeda, T., Narahashi, T., Casida, J.E.: α-Thujone (the active component of absinthe): g-Aminobutyric acid type A receptor modulation and metabolic detoxification. Proc. Natl. Acad. Sci. 97, 3826-3831 (2000). doi: 10.1073ypnas.070043297

46. Miyazawa, M., Watanabe, H., Kameoka, H.: Inhibition of Acetylcholinesterase Activity by Monoterpenoids with a *p* -Menthane Skeleton. J. Agric. Food Chem. 45, 677-679 (1997). doi: 10.1021/jf960398b

47. Mao; L., Henderson, G.: Evaluation of potential use of nootkatone against maize weevil (*Sitophilus zeamais* Motschulsky) and rice weevil [S. oryzae (L.)](Coleoptera: Curculionidae). Journal of Stored Products Research. 46, 129-132 (2010). doi: 10.1016/j.jspr.2010.01.002

48. Regnault-Roger, C., Hamraoui A.: Fumigant toxic activity and reproductive inhibition induced by monoterpenes on *Acanthoscelides obtectus* (Say) (coleoptera), a bruchid of kidney bean (*Phaseolus vulgaris* L.). Journal of Stored Products Research. 31, 291-299 (1995). doi: 10.1016/0022-474X(95)00025-3