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Insecticidal activity of ethanolic extract and essential oil of *Croton hirtus* L' Hér on *Amitermes evuncifer* Silvestri

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Abstract

Pests control has historically relied on the use of synthetic pesticides. Due to the recognized hazards these pesticides pose to both human health and the environment, there is a growing demand for alternative solutions such as the use of biopesticides. This study aims to assess the termiticidal efficacy of extracts derived from the plant Croton hirtus on the termites Amitermes evuncifer. The investigation also includes a phytochemical analysis of the ethanolic extract and a Gas Chromatography/Mass Spectrometry (GC/MS) analysis of the essential oil (EO) of C. hirtus. The termiticidal activity of C. hirtus extracts was evaluated through a contact approach involving A. evuncifer workers placed in Petri dishes. Five concentrations of the ethanolic extract and 4 concentrations of the essential oil were employed. The phytochemical analysis of the ethanolic extract of C. hirtus showed the presence of terpenes, reducing sugars, flavonoids, and sterols. Furthermore, by GC/MS analysis of the EO, caryophyllene (25.48%), germacrene-D (24.19%), β -elemene (8.25%), and α -humulene (7.69%) were identified. When tested, all concentrations of the extracts led to a reduction in the survival time and life expectancy of termites. Notably, the concentration of 4.7 mg/cm² of the extract resulted in a complete termite mortality rate within approximately 0.83 ± 0.26 days, while the concentration of $0.3 \,\mu\text{L/cm}^2$ of the essential oil caused a total mortality in approximately 2.50±0.55 days. These findings demonstrated that Croton hirtus extracts could be promising tools in the control of termites in an integrated pest management.

Keywords: Croton hirtus, Amitermes evuncifer, GC/MS, termiticide

Introduction

Termites are eusocial insects belonging to the infraorder Isoptera. They are one of the dominant species within the tropical ecosystem, and over a third of the world's species (Anani Kotoklo et al., 2010) ^[3] could be found in Africa. They feed on dead wood, but also on living plants and are often considered as ecosystem engineers (Martin, 2014) [22]. They play an essential role in nutrient recycling and are also involved in soil fertilization (Costa-Leonardo et al., 2002)^[9]. Nevertheless, about 5-8% of termite species cause damage to buildings, crops and forest resources (Wood, 1991)^[3]. In Africa, losses caused by termite pests are enormous and can often exceed 15%, sometimes even reaching 90% (Wood, 1991)^[3]. In Ivory Coast, some studies have been carried out on termite pest damage to yam, rice, and maize crops (Akpesse et al., 2008)^[2]. Amitermes evuncifer (A. evuncifer) is among the termite pests. This species has been found to be one of the termite pests of maize, cassava, wood, in Togo (Gbenyedji et al., 2014)^[14] and fruit trees in Senegal (Sane et al., 2016)^[28]. The control of termite pests often relies mainly on the use of synthetic insecticides. In the 1940s, pesticides of the organochlorine family, notably DDT (dichlorodiphenyltrichloroethane), were widely used in the control of insect pests (Levine, 2007)^[19]. The use of these synthetic insecticides has a detrimental effect on humans and the environment (Chèvre and Erkman, 2011)^[8]. Thus, in order to control insect pests without side effects, it is necessary to find other reliable and more environmentally friendly methods. The search for new substances such as plant extracts and essential oils have been promoted as biopesticides to provide crop protection and to contribute to the environmental protection.

Extracts and essential oils consist of secondary metabolites which are known to have different biological activities such as antioxidant, anti-inflammatory, insecticidal, antifungal, etc. Recently, studies have been carried out on the insecticidal activities of Ritchiea reflexa extracts (Adande et al., 2021)^[1] and its essential oil (Dossouvi et al., 2022) [11] on A. evuncifer. Other studies have also shown the effect of essential oils of Ocimum canum and Cymbopogon schoenanthus on termite pests like A. evuncifer (Gbenyedji et al., 2014)^[14]. It is in this perspective that Croton hirtus l'Hér an aromatic plant of the Togolese flora, about which few studies have been found in the scientific literature, caugh our attention. It is a plant of the Euphorbiaceae family with several traditional virtues. Moreover, studies have shown the presence of terpenoids, tannins, steroids and glycoside in the ethanolic extract of the plant (Subin and Reghu, 2012) [29]. Other studies on the essential oil showed the presence of E-caryophyllene as the main compound (Lima et al., 2012)^[34]. According to Tahiri et terpenoids have insecticidal activities and Eal.. caryophyllene has anti-termite activity (García et al., 2007) ^[13]. Thus, these compounds might have biological activities and could be used as bio- insecticide in the fight against insect pests. So, in this work we intended to develop botanical insecticides for the management of the termite species A. evuncifer using different concentrations of C. hirtus extracts.

Materials and Methods Plant material

The plant material consists of *Croton hirtus* aerial part, collected at Dalavé (Lat N 6 18'59,682" and Long E 1° 16'53, 4") located in the maritime region about 30 km from Lomé (Togo). It was identified from the Togolese flora and recorded by the herbarium of the Department of Botany of the University of Lomé under the number (TOGO 15882). This aerial part of the plant was dried before the different extraction methods.

Preparation of the C. hirtus extract

Two litters (2 L) of ethanol were added to 200 g of plant powder to obtain the maceration system. The mixture was subjected to intermittent manual agitation for 72 hours. The obtained filtrate maceration system was evaporated under vacuum at 45 °C using a rotary evaporator. The obtained extract was stored in a refrigerator.

Phytochemical screening

Phytochemical tests were carried out to determine the major chemical groups following the precipitation reaction and staining methods described by Kumar *et al.*,(2015) ^[15] and Rani *et al.*,(2017) ^[16].

Terpene test

Sulphuric acid is added to 1 mL of the extract solution. The mixture is heated in a water bath for 2 min. The presence of terpenes is indicated by the appearance of a grey color in the supernatant.

Flavonoid test

To 1 mL of the extract solution, 3 drops of 37% hydrochloric acid and a few milligrams of magnesium oxide are added. The red coloration indicates the presence of flavonoids.

Saponosid test

To 1 mL of the extract solution, 2 mL of distilled water is added. The mixture is stirred horizontally for 5 min. The formation of about 1 cm of foam indicates the presence of saponosides.

Reducing sugar test

To 1 mL of an extract solution, 2 mL of Fehling's liquor is added. The mixture is heated in a water bath for 8 min. The brick-red coloration indicates the presence of reducing sugars.

Cardiac glycoside test

Two milliliters of chloroform are added to 1 mL of the extract solution. Then we add two to three drops of a sulphuric acid solution to the mixture. The formation of the brown color indicates the presence of cardiac glycosides.

Test for tannins and phenols

To 1 mL of the extract solution heated in a water bath for 5 min at 60° C, 1 to 2 drops of a ferric chloride solution are added. The blackish-blue or greenish coloration shows that the result is positive.

Alkaloid test

To 1 mL of extract, Mayer's reagent (potassium mercuryiodide) is added. The formation of a yellowish-white precipitate indicates the presence of alkaloids.

Sterol and triterpene test

To 1 mL of the extract solution, a mixture of 0.5 mL of acetic anhydride and 0.5 mL of chloroform previously prepared is added. Concentrated sulphuric acid 37% is added to the previous mixture. The formation of the grey or violet color shows the presence of triterpenes and the formation of the blue to grey-green color shows the presence of sterols.

Quinine test

1% sodium hydroxide solution is added to 1 mL of the extract solution. The blue, green or red coloration indicates the presence of quinines

Isolation of essential oil

The essential oil of *C. hirtus* was extracted by steam training. A quantity of the aerial part previously dried in the laboratory for 5 days, was put in the distiller on a perforated grid located at a distance from the bottom of the distiller containing water. The water vapor produced by the water heating rises and passes through the biomass under the effect of pressure by dragging the volatile compounds to the condenser to give the hydrolate and essential oil, which will be obtained in a graduated burette.

GC/MS analysis

Gas Chromatographic analysis of essential oil was carried out on an Agilent Technologies 6850 chromatograph coupled to a 5973 mass selective detector, an automatic injector 7683B Series Injector. The injection was performed in splitless mode. The carrier gas was helium with a flow rate of 1 mL/min and 1µL of the sample was injected. The column used was apolar 5% phenylmethylpolysiloxane (30 m x 0.25 mm; film thickness = $0.25 \,\mu$ m). The injector temperature was maintained at 250 °C. The oven temperature was programmed as follows: from 50 to 230 °C (5 °C /min) in 36 min and maintained isothermal for 2 min. The GC/MS is connected to a computer system with a NIST98 mass spectrum library and controlled by "XCALIBUR" software to monitor the progress of the chromatographic analyses. The identification of the constituents was done by comparing their retention indices with those of the computer system standard.

Bioassay

Amitermes evuncifer Silvestri (Blattodea, Termitinae) worker termites were used for the biological tests. They were acclimatized under laboratory conditions (28°C, 89% relative humidity) in total darkness within 24 hours or a day (12H: 12H DD). However, the tests were carried out according to the method of Raina *et al.*, (2012) ^[24].

Termiticide test of ethanolic extract on A. evuncifer

Two controls (negative and positive) were used for the tests. The negative control consists of the 9 cm diameter plastic Petri dish and a slice of filter paper. The positive control consists of the same as the negative control; except that the filter paper is impregnated with the extraction solvent (ethanol) and left in the open air for complete evaporation of the solvent for 24 hours. Five concentrations were used for the tests: 0.4; 0.8; 1.6; 3.2 and 4.7 mg/cm². To obtain them, masses of extract were each dissolved in 5 mL of ethanol directly in the petri dish on the filter paper (9 cm in diameter). The whole set was left in the open air for 24 hours to allow total evaporation of the solvent. Six replicates were performed for each concentration including the controls (negative and positive). Thirty workers of A. evuncifer were exposed to each of the previously prepared concentrations in Petri dishes. The Petri dishes were placed in a tray in total darkness (12H: 12H DD) under laboratory conditions (28 °C and 89% relative humidity). The termites were then checked every hour for 6 hours and after the sixth hour, the check was performed after every 24 hours until the death of the last worker. At each check, live and dead termites were counted and the dead ones were removed from the Petri dishes.

Termiticide test of the essential oil on A. evuncifer

Two controls were used (negative control and positive control). The negative control is a filter paper disc placed in the petri dish. The positive control is made up of a slice of filter paper soaked in 5 mL of a hexane solution and then left under a fume hood for the hexane to evaporate completely. Four concentrations of the essential oil namely 0.1; 0.15; 0.19 and 0.3 μ L/cm² were used. Respective volumes of each concentration were initially dissolved in 5 mL of hexane to obtain the different concentrations. The resulting mixture was poured into a glass Petri dish (3.5 cm radius) containing a filter paper disc. The whole was placed in a fume hood for 3 hours to allow the hexane to evaporate completely. The prepared concentrations were then stored in a refrigerator (5-10 °C) for 24 hours before the start of the bioassay.

Twenty workers of *A. evuncifer* were placed in contact with the previously prepared controls and concentrations. Six replicates were made for each test and the whole set was placed in a dark tank (12H: 12H DD) under laboratory conditions (28 °C and 89% relative humidity). Checks were then made every hour for the first six hours and then after every 24 hours until the last termite died. Dead and living individuals were counted and the dead were removed from the Petri dishes.

Data analysis

Survival time is the time it takes for termite individuals to die after contact with the different concentrations tested (Krebs 1999)^[18].

Ds = Tx - To

Ds: survival time

Tx: time taken by the last individual of termite to die *To*: initial time of contact of termites with different concentrations

The survival proportion and life expectancy were calculated with Ecological Methodology 7.3 (Krebs, 1999) ^[18]. The survival proportion is the ratio of the number of termites alive at each test time to the number of termites initially in contact with the test concentrations. Life expectancy is an estimate of the number of days that a termite individual in contact with the extracts is expected to live before dying.

One-way analysis of variance (One way ANOVA) was used to discriminate the means of survival time and life expectancy of termites within the concentrations of each extract (ethanolic extract and essential oil) of *C. hirtus* with the LSD test (Least Significant Difference). The analysis was performed with SPSS 20 software.

Results and Discussion Phytochemical screening

The phytochemical study performed on the ethanolic extract showed the presence of terpenes, reducing sugars, flavonoids and sterols (Table 1). Nevertheless, Subin and Reghu ^[12], in India found in the ethanolic extract of the plant the presence of tannins, steroids, terpenoids and glycosides. The results we obtained will allow us to demonstrate the insecticide activity of the plant based on the difference of phytochemicals. Thus, terpene insecticidal activities were reported by Rossi *et al.*, (2012) ^[27]. Madaci *et al.*, (2008) ^[21] also showed that flavonoids have antibacterial and insecticidal activity. Sterols are known to reduce cholesterol in blood plasma (Katan *et al.*, 2003) ^[16]. These different large chemical groups can allow us to demonstrate several biological properties of the plant.

 Table 1: Phytochemical test results for the ethanolic extract of C.

 hirtus.

Tests	Ethanolic extract
Terpenes	+
Saponosids	-
Tannins	-
Reducing sugars	+
Phenols	-
Alkaloids	-
Flavonoids	+
Cardiac glycosides	-
Quinines	-
Sterols	+
Triterpenes	-

(+): Presence of active substance, (-): absence of active substance

GC-MS analysis of the essential oil of C. hirtus

The chemical composition of the essential oil of the aerial part of C. hirtus, according to the chromatogram, showed four dominant peaks (Figure 1). A total of forty-one (41) compounds were identified (Table 2) with a total content of 100%. Among these 41 compounds, four are majority including β -elemene (8.25%), caryophyllene (25.48%), germacrene-D (24.19%), and α -humulene (7.69%). These four main components are sesquiterpene hydrocarbons. However, in Ivory Coast, the essential oil of the leaves identified by GC-MS showed the predominance of (E)caryophyllene (31.75%), germacrene-D (22.57%) and α humulene (7.42%) and the predominance of (E)-(9.64%) (37.72%), α-humulene caryophyllene and caryophyllene oxide (6.95%) in the stems (Daouda et al., 2014) ^[10]. According to Hanane, (2020) ^[15] essential oils

constitute new potential sources of control of crop pests because they are very rich in bioactive molecules with insecticidal properties. Indeed, caryophyllene and germacrene-D, the compounds with the highest percentage in the essential oil, have been reported to be among the essential oils with a high degree of anti-termite properties (García *et al.*, 2007) ^[13]. Benelli *et al.*, (2018) ^[5] reported that α -

humulene has insecticidal and anti- inflammatory activity (Fernandes *et al.*, 2007)^[12]. As for β -elemene, it is used as an anti-cancer ingredient (Wang *et al.*, 2022)^[32]. Therefore, the existence of caryophyllene, germacrene-D, β - elemene and α -humulene in the essential oil of the plant may be responsible for the presence of several properties in the plant.

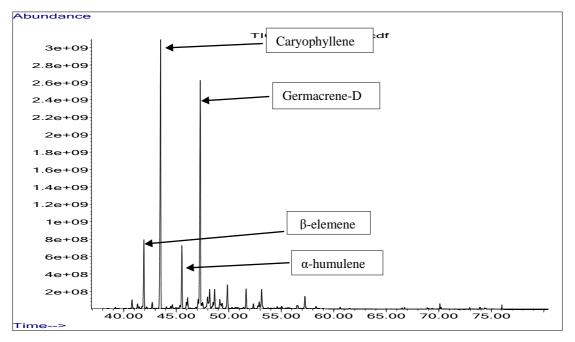


Fig 1: Chromatogram of the essential oil of the aerial part of C. hirtus

Table 2:	Chemical	compos	ition of	of <i>C</i> .	hirtus	essential o	il
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\mathbf{N}°	RT	Components	%HE	N°	RT	Components	%HE
1	29.935	Decanal	0.07	22	48.658	δ-Guaiene	2.66
2	39.128	Longicyclene	0.20	23	49.151	β-Curcumene	1.77
3	40.002	Cyclosativene	0.05	24	49.369	Cubebol	0.93
4	40.516	β-Elemene	0.08	25	49.875	δ-Cadinene	3.55
5	40.791	Isoledene	1.14	26	51.396	Elemol	0.32
6	41.308	β-Bourbonene	0.61	27	51.647	Germacrene B	2.53
7	41.920	β-Elemene	8.25	28	52.341	E-Nerolidol	0.77
8	42.165	Cyperene	0.30	29	53.134	Spathulenol	3.11
9	42.716	Sativene	0.80	30	54.627	Humulene-1,2-epoxide	0.42
10	43.478	Caryophyllene	25.48	31	55.052	Himbaccol	0.46
11	44.063	Cedrene	0.27	32	56.504	Cedrelanol	0.86
12	44.488	γ-Elemene	0.36	33	57.252	α-Cadinol	2.12
13	44.631	α-Bergamotene	0.56	34	58.320	Cadalene	0.61
14	45.362	α-Himachalene	0.52	35	60.616	1-Pentadecanal	0.22
15	45.529	α-Humulene	7.69	36	61.494	Mintsulfide	0.15
16	46.073	trans-β-Farnesene	1.36	37	66.517	Neophytadiene	0.13
17	47.083	γ-Muurolene	1.31	38	68.898	Cembrene	0.21
18	47.260	Germacrene D	24.19	39	73.921	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl	0.41
19	47.519	β-Selinene	0.86	40	74.401	Gamolenic acid	0.42
20	48.182	Bicyclogermacrene	3.10	41	76.010	Phytol	0.30
21	48.508	α-Muurolene	0.86			Total	100

RT: retention time

Termiticidal activity of ethanolic extract of C. hirtus

Effect of ethanolic extract on survival time of *A. evuncifer* The concentrations of the ethanolic extract affected the survival time of *A. evuncifer*. Ethanolic extract concentrations affected the survival time of *A. evuncifer*. One-factor analysis of variance showed a significant difference between concentrations ($F_{(6.35)} = 49.345$, p < 0.001) and controls (Table 3). The reduction was significant between 0.4 and 0.8 mg/cm². The survival times were 13.00 ± 0.89 and 6.33 ± 1.51 days respectively. No significant difference was found between the concentrations 0.8 and 1.6 mg/cm² with survival times of 6.33 ± 1.51 and 3.83 ± 1.94 days respectively. Similarly, no significant difference was found between the concentrations 1.6, 3.2, and 4.7 (3.83 ± 1.94 , 1.25 ± 0.61 , and 0.83 ± 0.26 days respectively) (p = 1, LSD). The compounds, such as terpenes or flavonoids (Madaci *et al.*, 2008; Rossi *et al.*, 2012) ^[21, 27], might affect by termites by reducing their survival time, as in the case of pyrethrum, extracted from the flower of *Tanacetum cinerariifolium* or chrysanthemum, which has a very effective insecticidal activity (Anjarwalla *et*

al., 2016) ^[4]. Indeed, some studies have shown that plants possess repellent and toxic activities against insect pests (Regnault-Roger and Hamraoui, 1993) ^[26]. So, ethanolic

extracts of *Ritchiea reflexa* and *Ctenium elegans* have been found harmful to *A. evuncifer* termites in Togo (Adande *et al.*, 2021)^[1].

Table 3: Survival time of A	evuncifer workers in contact with ethanolic extract of C.	hirtus
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Concentrations	Survival time (days)				
Concentrations —	Ethanolic extract				
Negative control	27.33±6.56 ^a *				
Positive Control	27.33±5.54ª				
0.4 mg/cm ²	13.00±0.89 ^b				
0.8 mg/cm ²	6.33±1.51°				
1.6 mg/cm^2	3.83±1.94 ^{cd}				
3.2 mg/cm^2	1.25 ± 0.61^{d}				
4.7 mg/cm ²	0.83 ± 0.26^{d}				
	$F_{(6.35)} = 49.345; p < 0.001$				
	LSD post hoc test				

* Averages followed by the same letter are not significantly different

Effect of ethanolic extract on the survival rate of A. evuncifer

All concentrations of ethanolic extract tested affected the survival ratio of *A. evuncifer* as well as the survival time. The survival ratio in the concentrations 0.4 mg/cm² and 0.8 mg/cm² exhibited in Figure 2 decreased gradually (0.99±0.01 and 0.99±0.01 respectively) from third hours (0.13 days) and fourth hours (0.17 days) during the first 6 hours of the test until fifteenth and ninth days respectively when all termites died. This decrease was fast in high concentrations 3.2 mg/cm² and 4.7 mg/cm². So, the decrease could be attribute to the presence of terpenes and flavonoids (Madaci *et al.*, 2008; Rossi *et al.*, 2012) ^[21, 27] in the ethanolic extract of the plant.

Effect of ethanolic extract on life expectancy of A. *evuncifer*

The ethanolic extract of the plant also significantly reduced

the life expectancy of termites (F $_{(6.35)}$ = 49.345; p<0.001) compared to controls. No significant difference was observed between the life expectancy durations of the negative and positive controls $(14.64\pm1.47 \text{ and } 14.67\pm1.92 \text{ days})$ respectively). Compared to the other concentrations, the concentrations 0.4 and 0.8 mg/cm² were less effective (12.33±0.70 and 9.91±0.89 days respectively). However, concentrations of 1.6, 3.2 and 4.7 mg/cm² of the ethanolic extract of C. hirtus were effective in reducing the life expectancy of A. evuncifer (7.69±1.17, 6.57±0.39 and 6.27±0.39 days respectively Figure 3). Moreover, effects of concentrations on survival time and life expectancy of termites increased with increasing concentration (Table 3 and Figure 3). According to Gbenyedji et al., (2014) [14] the effectiveness of plant extracts depends on the concentrations used on termites. Indeed, the efficiency of our plant could be related to the presence of terpenes in the ethanolic extract which have insecticidal activity (Rossi et al., 2012)^[27].

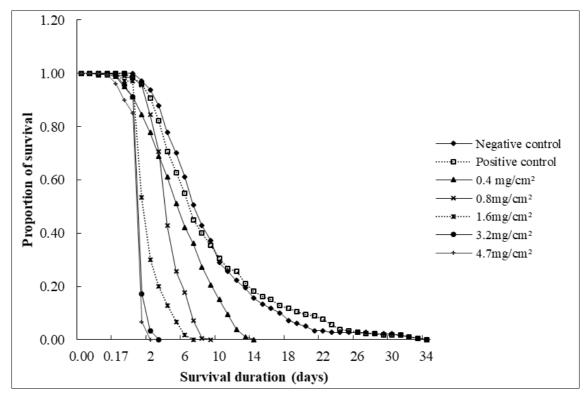


Fig 2: Proportion of survival of termites in contact with the ethanolic extract of Croton hirtus

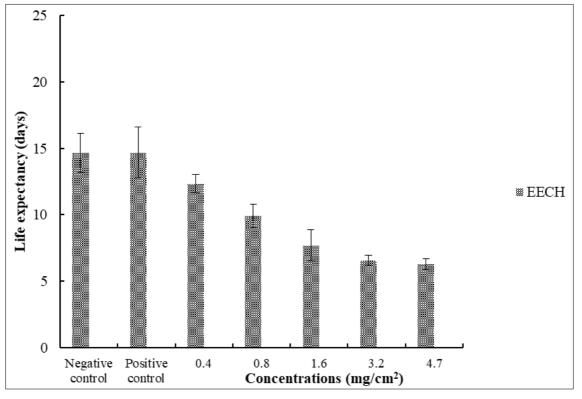


Fig 3: Life expectancy of termites in contact with the ethanolic extract of Croton hirtus

Termiticidal activity of *C. hirtus* essential oil Effect of essential oil on survival time of *A. evuncifer*

All concentrations of the essential oil tested significantly reduced survival time (F $_{(5.30)} = 74.562$; p < 0.001) compared to negative and positive controls. However, no significant difference was observed between the concentrations tested (p = 1, LSD post hoc test) (Table 4). More, the perfomed bioassays showed the effectiveness of the concentrations. This

anti- termite activity of *C. hirtus* may also be due to caryophyllene and germacrene-D, which exhibit among the essential oils components ones with a high degree of antitermite properties (García *et al.*, 2007) ^[13]. Nevertheless, it is difficult to attribute the anti-termite biological activity only to the main compounds in the essential oil alone, as a synergistic effect could take place (Bouhdid *et al.*, 2012) ^[6].

Concentrations	Survival time (days)				
Concentrations	Essential oil				
Negative control	27.33±6.56 ^a *				
Positive Control	27.17±5.67ª				
$0.1 \ \mu L \ /cm^2$	3.33±0.52 ^b				
$0.15 \mu L /cm^2$	2.83±0.75 ^b				
$0.19 \mu L/cm^2$	2.50±0.84 ^b				
$0.3 \mu L /cm^2$	2.50±0.55 ^b				
	$F_{(5.30)} = 74.562; p < 0.001$				
	LSD post hoc test				
	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1				

Table 4: Survival time of A. evuncifer after contact with the essential oil of C. hirtus

* Averages followed by the same letter are not significantly different

Averages followed by the same letter are not significantly different Effect of essential oil on the survival rate of *A. evuncifer*

The survival ratio curves show that termites in the controls had a longer survival time than those in the different essential oil concentrations (Figure 4). All concentrations reduced the survival rate of the termites. A more or less fast decrease was observed for the tested concentration. From the first hour (0.04 day) and second hour (0.08 day), there was a gradual decrease in the survival ratio in the concentrations $0.1 \,\mu\text{L/cm}^2$ and $0.15 \,\mu\text{L/cm}^2$, respectively (0.98 ± 0.03 and 0.98 ± 0.02 , respectively) until the fifth day. Also, in the first 6 hours of the bioassays, the decrease in survival proportion started at first hour ($0.04 \,\text{day}$) in the high concentrations $0.19 \,\mu\text{L/cm}^2$ and $0.3 \,\mu\text{L/cm}^2$ (0.98 ± 0.03 and 0.92 ± 0.07 , respectively) until fifth and fourth day, respectively (Figure 4). We observe that

as the concentration increases, the efficiency also increases. According to Hanane, (2020), the insecticidal activity of an essential oil varies according to the concentration and the duration of the treatment. Thus, this effectiveness would be due to the high content of caryophyllene (25.48%) whose anti-termite activity has been demonstrated as a defensive substance against *Coptotermes formosanus* (Cheng *et al.*, 2004) ^[7]. This could also be due to the synergistic effect between the main constituents and other compounds identified in the essential oil.

Effect of essential oil on life expectancy of A. evuncifer

The essential oil also affected the life expectancy of the termites (F $_{(5.30)} = 74.562$; p < 0.001) compared to both negative and positive controls (14.64±1.47 and 15.05±1.60 days, respectively Figure 5). All tested concentrations were

effective. No significant difference was observed between the calculated life expectancy values for the different concentrations 0.1; 0.15; 0.19 and 0.3 μ L/cm² (8.48±0.33, 7.76±0.67, 7.61±0.51, and 6.83±0.95, respectively). Thus this

reduction in life expectancy of *A. evuncifer* could be due to the synergistic or individual effect of the different molecules identified in the essential oil (Park & Shin, 2005)^[23].

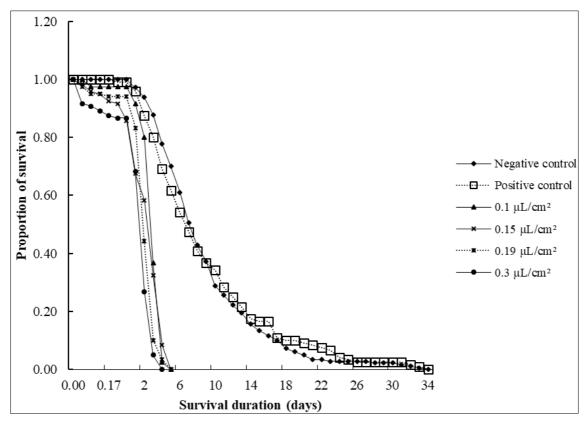


Fig 4: Survival proportion of termites in contact with *Croton hirtus* essential oil.

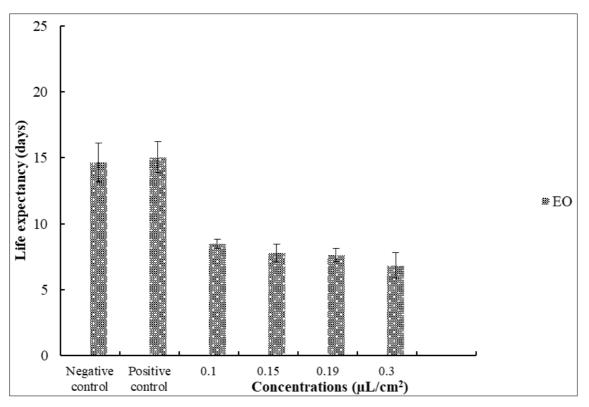


Fig 5: Life expectancy of termites in contact with Croton hirtus essential oil

Conclusion

The phytochemical study that we carried out on the ethanolic extract showed the presence of terpenes, reducing sugars, flavonoids and sterols. The GC/MS analysis exhibited forty-

one (41) compounds of which main were sesquiterpene hydrocarbon compounds, namely caryophyllene (25.48%), germacrene-D (24.19%), β -elemene (8.25%), and α -humulene (7.69%). According to the bioassays, all tested concentrations

of the ethanolic extract and essential oil reduced the survival time, survival ratio, and life expectancy of the termites. The ethanolic extract and essential oil of *Croton hirtus* possess insecticidal activities that might be used as a bio-insecticide in the management of *A. evuncifer* (termite pest) species.

Conflict of interest

The authors declare that they have no conflict of interest.

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