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Composition and termidicidal activity of essential oils of *Ritchiea reflexa* (Thonn.) Gilg & Benedict and *Ctenium elegans* Kunth. against *Amitermes evuncifer* Silvestri, 1912

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Leaves of *Ritchiea reflexa* (Thonn.) Gilg & Benedict and aerial parts of *Ctenium elegans* (Kunth, 1829) were steam distillated to obtain their respective essential oils. Gas chromatography-mass spectrometry was used to characterize essential oil components. The susceptibility of these volatile oils on *Amitermes evuncifer* (Silvestri, 1912) was tested using contact test with washer of filter paper inside Petri dish. Twenty (20) workers of *A. evuncifer* were put in contact with 4 different doses of each tested essential oil. The doses were compared to two controls (negative and positive). The mortality of termites was recorded daily until the death of the last individual. GC-MS data showed that *R. reflexa* oils contained 3 components while that of *C. elegans* contained 14 compounds. Benzyl cyanide (> 80%), Benzyl isothiocyanate (> 4%), and Diisooctyl phthalate (8.98%) were the three components in *R. reflexa* essential oils. α-Cadinol (21.43%), Elemol (16.27%), Methyl (2Z)-3-(2,2,6-trimethyl-5-oxo-7-oxabicyclo[4.1.0]hept-1-yl)-2-propenoate (10.67%), δ-Cadinene (8.46%), and τ-Muurolol (7.56%) were the main components of *C. elegans* essential oil. Oxygenated sesquiterpenes (58.6%) and sesquiterpene hydrocarbons (24.55%) were then the two main groups of volatile oil components of *C. elegans*. All the tested doses significantly reduced the survival duration and life expectancy of *A. evuncifer* in comparison to controls. However, the effect of essential oils was dose dependent: the highest doses (0.19 and 0.30 µl/cm^2) killed all the termites one hour after the contact (for *R. reflexa*). It clearly appeared that these oils significantly affected *A. evuncifer*. Thus, they could be regarded as potential alternatives to synthetic chemicals.

Key words: *Ritchiea reflexa*, *Ctenium elegans*, essential oils, termidicidal activity, *Amitermes evuncifer*.

INTRODUCTION

The use of plants is a cultural component of human beings. Human has taken advantage of plants to meet his needs. The plants benefits are extremely wide and various. Aromatic plants constitute an important group of

tropical plant resources. They present considerable advantages due to the progressive discovery of essential oil's applications in health care and in many other areas of economic interest like perfumes, cosmetics, soap, shampoos, cleaning gels, and agrofood industry (Ríos, 2016; Ali et al., 2015). The current esteem of essential oils and aromatic plants was due to their anti-inflammatory, antiseptic, antiviral, antifungal, bactericidal, antitoxic, insecticidal, insect repellant, stimulating, and calming properties (Ríos, 2016; Ali et al., 2015; Mishara and Dubey, 1994; Tellez et al., 2000; Gkinis et al., 2003). As part of the study contribution to developing tropical aromatic plants, we have been particularly interested in *Ritchiea reflexa* and *Ctenium elegans* essential oils' pesticidal effects. Indeed, *Ritchiea* is the most widespread genus of Capparaceae family in dense humid forest. The species *R. reflexa* is traditionally used to treat migraines (Letouzey, 1982). However, little is known about the secondary constituents of *R. reflexa*. On the other hand, the genus *Ctenium* belongs to Gramineae family, the most important to humans with its many cereals (rice, corn, sorghum, and fonio; 500 genera with 8000 cosmopolitan species). The species *C. elegans* is a rhizomatous hardy perennial herb from Africa savanna (Thiombiano, 2012) and is traditionally used to manufacture roofs and thick mats by interweaving stems (Brink and Achigan-Dako, 2012). Moreover, cattle breeders after successfully treating unexploited leaves and young stems with urea, use the resulting fodder to feed animals (Brink and Achigan-Dako, 2012). There is also a very limited literature on the secondary constituents of *C. elegans*.

Termites are not only pests of agriculture and forestry affecting woods and crops, but also structural pests causing damage to wooden structures, household furniture, books, and museum collections (Govorushko, 2018). Pestiferous termite such as *Amitermes evuncifer*, can cause heavy damage to plants and agricultural crops (Govorushko, 2018; Loko et al., 2017). However, along with ants and earthworms, termites are one of the three main groups of soil ecosystem engineers (Lavelle et al., 2006). More, termites, like ants and honeybees, are social insects (Govorushko, 2018; Loko et al., 2017). As that, termites can be used as indicators of various environmental features, such as anticipated rainfall and soil fertility (Govorushko, 2018). It is therefore required making termites management environmentally and ecologically friend for the benefice of biotopes. Conventional termiticides (Loko et al., 2017) are identified as harmful persistent organic pollutants (UNEP, 2008). The today focus is the control of termites using non-synthetic chemicals, notably bio-pesticides (Verma et al., 2009; Gregory et al., 2014) in a system of integrated pest management with bait trials (Su, 2019). The bait trial consists of attracting termites with a good attractant (commonly a rich cellulose material) in a monitoring point. Once the bait is infected, the chemicals is placed inside the monitoring point in order to infest harvesting workers of termites that will carry and bring the infestation back to the nest and contaminate the whole colony. Besides the finding of a good attractant, another constraint of this strategy is to find out the suitable concentration of a non-repellent contaminant product that, instead of killing termite *on situ*, will affect their survival duration and life expectancy. Therefore infested (but still alive) workers will bring the contaminant within the nest and infest the whole members of the colony leading to its total and complete death.

The termiticidal propriety of volatile oils or their constituents have been reported (Tellez et al., 2002; Sakasegawa et al., 2003; Roszaini et al., 2013) and constitute now a credible option for best pest's management. To the best of our knowledge, the composition and the termidicidal activity of the volatile oils of *R. reflexa* and *C. elegans* has not been previously reported and constitute the aim of this paper. Thus, in this study, we evaluated the antitermite activity of three formulations of essential oils of *R. reflexa* and *C. elegans* against *A. evuncifer* in order to find out the suitable concentration for baiting trial.

MATERIALS AND METHODS

Plant

An amount of 2.855 kg of the fresh aerial parts of *R. reflexa* was collected from Kpala (Lattitude 6°16'21.59292" N and Longitude 1°6'49.15152" E), near Lomé in Togo. Similarly, 1.342 kg of the dried materials of *C. elegans* was harvested at Danyi Koudzragan (Lattitude 7°8'29.1768" N and Longitude 0°37'30.21996" E), a mountainous zone in Togo. Plant species were identified in the herbarium of "Université de Lomé", and registered under the numbers of TOGO-15508 for *R. reflexa* and TOGO-15509 for *C. elegans*. Voucher specimens were placed in the herbarium unit for future references. The foreign materials in the plant samples were carefully removed, and a part of *R. reflexa* fresh materials was air dried for about 20 days at room temperature. Only the leaves were steam-distilled for *R. reflexa*, while the whole aerial part was concerned for *C. elegans*.

Isolation of essential oils

Essential oils were steam-distilled in a modified laboratory-scale Clevenger-type apparatus with the total collected amount of each plant species. About 250 g of plant biomass were loaded into a still for a volume of 5 L of water. Each distillation was monitored for about 3 h. This operation was repeated until there was no plant material left. The collected essential oils were dried over anhydrous sodium sulphate, placed for 48 h in a vacuum desiccator for

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complete removal of water traces, and stored in vials at about 0°C until required.

GC-MS analyses

The chromatographic separation of the metabolites for component identification purposes was performed on an Agilent Technologies 6850 gas chromatograph coupled with a mass detector 5973 and a 7683B series injector autosampler, and the injection was performed in splitless mode. The resulting data were elaborated using MSD ChemStation. The column was 5% phenylmethylpolysyloxane (30 m × 0.25 mm; film thickness = 0.25 µm). Injector temperature was kept at 250°C. The oven temperature was programmed as follows: 50°C for 10 min and a ramp from 50 to 300°C (10°C/min in 25 min) and kept at this temperature for 4 min. The carrier gas was helium with a flow of 1 mL/min, and 1 µL of the sample was injected. The mass detector settings were as follows: ionization voltage, 70 eV; scan rate, 2.91 scan/s; mass range, 50-550; transfer line, 230°C. The components of the samples were identified by (a) comparison of their relative retention times and mass fragmentation with those of authentic standards and (b) computer matching against NIST98, as well as linear retention indices calculated using C_9−C_{24} alkanes compared with those reported by Adams (2009). Quantitative analysis of each component was carried out with an external standard method when available.

Termiticidal assays

Termites sampling and accommodation

Termiticidal activity of essential oils from the aforementioned plant species were tested against *A. evuncifer* Silvestri, 1912 (Blattodea, Termitinae). This species is one of the most important termite pests that attack and damage not only a wide range of food crops such as yam, groundnut, manioc sugar cane and maize, but also ligneous plants at different age as well as furniture inside habitations (Umeh and Ivbijaro, 1997; Sands, 1973). All the tested individuals of *A. evuncifer* were collected from the same nest located inside the botanical garden of the “Université de Lomé” (Togo). Collected termites were accommodated to the conditions of the laboratory (28°C and Relative Humidity of 90% at total darkness 12:12 DD) 24 h previous to bioassay.

Bioassay

Biological assays were carried out by contact according to Pearce (1997). Four different concentrations (µl/cm^2) were tested against *A. evuncifer*: 0.1, 0.15, 0.19 and 0.3. To get these concentrations, a corresponding volume of each tested dose from each essential oil was initially dissolved in 15 ml of hexane. The obtained solution was poured inside a Petri dish (7 cm diameter) that contained a washer of filter paper (6.8 cm diameter) at its bottom. The soaked washer of filter paper was then left under hood for 3 h in order to evaporate all the hexane and keep the essential oil evenly impregnated. The prepared washers were kept at 5 to 10° during 24 h previous to bioassays.

To see whether the solvent (hexane) had an effect on termites, two controls were made: negative and positive. For negative control, washer of filter paper (6.8 cm diameter) without any treatments was used. In positive control, the washer of filter paper was previously soaked with 15 ml of hexane and kept under hood for total evaporation.

Twenty healthy and vigourous workers of *A. evuncifer* were put in contact with each previously prepared washers of filter paper inside Petri dishes. Petri dishes (containing washer of filter paper and termites) were covered with lids and kept under controlled conditions of temperature (28°C) and relative humidity (90%) at total darkness (12:12 DD). A total of six replicates were made for each concentration as well as negative and positive controls. Observations were done after each hour during the first 6 h. After the 6 h and if termites were still alive, observations were done after each 24 h until the death of the last individual of each Petri dish. At each observation time, the number of alive and dead termites was recorded. Dead termites were thoroughly removed from the Petri dish and only alive individuals were kept.

Data analysis

As the main objective was to see the effect of tested essential oils on the survival of termites, so termites were observed until the death of the last individual. Therefore, analyses were focused on the survival duration, the proportion of individuals that survive after each checking time and the life expectancy of surviving individuals after the contact with each concentration of the essential oil. Values of both survival proportion and life expectation were means obtained by averaging data from six replicates.

The proportion of survival was calculated following the formula given by Krebs (1999):

$$L_x = {}^{n_x}/_{n_0}$$

Where L_x: proportion of individual surviving at the start of time x, n_x: number of individual alive at the start of time x, and n_0: number of initial individual alive at the starting time (0).

The proportion of survival showed how fast termites died and how long they survived in contact of tested concentrations.

Life expectancy was also calculated following the formula from Krebs (1999):

$$L_x = \frac{n_x + n_{x+1}}{2}$$

Where Lx: mean number of individual alive during the time interval x to x+1 and n^{x+1}: number of individuals alive at start of time x+1.

$$Tx = \sum_{i=x}^{m} Li$$

Where T_x: cumulative of individuals alive between time x and m, Li: mean number of individual alive at time I, and m: maximum time reached (observed).

$$E_x = {}^{T_x}/_{n_x}$$

Where E_x: mean expectation of further life for individuals alive at start of time x.

To analyze the difference within both the survival duration and the lives expectation of three types of essential oil, two-ways analysis of variance (ANOVA) was used with Bonferroni post hoc test. Because of the significant difference observed between the types of essential oil, one-way ANOVA was run between concentrations within the same type of essential oil. All analyses were performed with SPSS 20 software.

RESULTS

Essential oil characteristics

The average yields of the essential oils of *R. reflexa*

Table 1. Chemical composition of *R. reflexa* essential oils.

Peak[a]	RI[b]	LRI[c]	Area percentage[d] (%)		Components
			Fresh leaves	Dry Leaves	
1	1148	1152	82.47	95.5	Benzyl cyanide
2	1389	1380	8.51	4	Benzyl isothiocyanate
3	2741	2695	8.90	-	Diisooctyl phthalate

[a]Elution order on a DB-5MS column; All compounds were identified by mass spectra, GC retention indices, comparison with literature data and co-injection with authentic compounds. [b]Retention index as reported by Adams (2009). [c]Linear retention indices as calculated according to Kovats (1978) for alkanes C9-C24. [d]Mean value of three determinations (three replicates) calculated from GC-MS areas.
Source: Author

Table 2. Chemical composition of *C. elegans* essential oil.

Peak[a]	RI[b]	LRI[c]	Area (%)[d]	Compounds
1	1148	1152	6.16	Benzyl cyanide
2	1391	1403	2.21	β-Elemene
3	1418	1444	3.22	Caryophyllene
4	1436	1445	4.34	β-Guaiene
5	1491	1475	4.58	Valencene
6	1524	1537	8.46	δ-Cadinene
7	1567	1560	6.27	Globulol
8	1580	1570	1.76	Epiglobulol
9	1555	1571	16.27	Elemol
10	1619	1596	5.31	τ-Eudesmol
11	1642	1642	7.56	τ-Muurolol
12	1644	1645	1.74	α-Amorphene
13	1640	1666	21.43	α-Cadinol
14	-	1663	10.67	Methyl (2Z)-3-(2,2,6-trimethyl-5-oxo-7-oxabicyclo[4.1.0]hept-1-yl)-2-propenoate

[a]Elution order on a DB-5MS column; All compounds were identified by mass spectra, GC retention indices, comparison with literature data and co-injection with authentic compounds. [b]Retention index as reported by Adams (2009). [c]Linear retention indices as calculated according to Kovats (1978) for alkanes C9-C24. [d]Mean value of three determinations (three replicates) calculated from GC-MS areas.
Source: Author

leaves were 0.10 and 0.04% (v/w) for fresh and dry biomasses, respectively. These oils were found very strong in smell. The obtained yield was low (0.006%) for *C. elegans*. The characteristics of oils' chemical composition are shown in Tables 1 and 2. In total, three compounds were identified in essential oils of *R. reflexa* (all three in fresh leaves and only two in dry leave oil samples). The total content of the two or three compounds accounted for more than 99.00%. Among these phenylpropanoid derivatives was 1 nitrogen with very notable content (benzyl cyanide, 82.47 and 95.5%), 1 nitrogen and oxygen (benzyl isothiocyanate, 8.51 and 4%) and 1 oxygen (Diisooctyl phthalate, 8.91%) which was only obtained in fresh leaves oil sample.

On the other hand, *C. elegans* essential oil gave fourteen compounds accounting for 99.98%. All those compounds were mainly sesquiterpenes except the benzyl cyanide which is a phenylpropanoid. Six compounds were sesquiterpene hydrocarbons (24.55%), six other were oxygenated sesquiterpenes (58.6%), one acid oxygenated unsaturated bicyclic compound (10.67%), and one phenylpropanoid derivative (benzyl cyanide, 6.17%). The main constituents in the oil were then α-cadinol, elemol, methyl (2Z)-3-(2,2,6-trimethyl-5-oxo-7-oxabicyclo[4.1.0]hept-1-yl)-2-propenoate, all oxygenated derivatives, followed by δ-cadinene and τ-muurolol. Alpha-Cadinol is a sesquiterpenoid alcohol which had been found to be anti-fungal and hepatoprotective (Ho et al., 2011; Tung et al., 2011).

Survival duration of *A. evuncifer*

All the tested concentrations of the three essential oils affected the survival duration of *A. evuncifer* (Table 3). Two-way ANOVA showed that there was not only a significant difference between the three essential oils ($F_{(2,105)} = 12.762$, $p < 0.001$) and concentrations

Table 3. Survival duration (Mean±SD) of *A. evuncifer* after the contact with essential oils.

Parameter	Survival duration (h)		
	C. elegans	***R. reflexa* (FL)**	***R. reflexa* (DL)**
Negative control	796±104.52[a]	796±104.52[a]	796±104.52[a]
Positive control	704±116.26[a]	704±116.26[a]	704±116.26[a]
0.1 µl/cm²	44±23.60[b]	534±30.2[b]	124±48.9[b]
0.15 µl/cm²	40±12.39[b]	4.17±2.04[c]	1[c]
0.19 µl/cm²	18±9.3[b]	1[c]	1[c]
0.3 µl/cm²	9[b]	1[c]	1[c]
	$F_{(5,30)}$ = 199.446; *p<0.001* Bonferroni post hoc test	$F_{(5,28)}$ = 190.559; *p<0.001* Bonferroni post hoc test	$F_{(5,30)}$ = 189.122; *p<0.001* Bonferroni post hoc test

Data (Mean±SD_ within the same column, followed by the same letter are not significantly different (p>0.05). FL: Fresh leaves; DL: dry leaves.
Source: Author

($F_{(5,105)}$=549.429, *p<0.001*), but also an interaction between these essential oils and the concentrations ($F_{(10,105)}$=12.961, *p<0.001*). Thus, one-way ANOVA was used to analyze each essential oil separately.

For the essential oil of *C. elegans*, all the tested concentrations significantly reduced the survival duration ($F_{(5,30)}$=199.446, *p<0.0001*), compared to the controls (Table 3). No significant difference was found between these concentrations in term of reduction of survival duration (p=1, Bonferroni post hoc test). For essential oil of the fresh leaves of *R. reflexa*, although all the concentrations significantly reduced the survival duration of termites ($F_{(5,28)=}$190.559*, p<0.001*), the effect seemed to depend on the concentration (Table 3). Indeed a significant difference was found between the lower concentration (0.1 µl/cm²) and the three others whose survival durations were not significantly different (p=1 Bonferroni post hoc test). The same pattern was observed for the essential oil of the dry leaves of *R. reflexa*. For this essential oil, all the concentrations significantly affected the survival duration of termites ($F_{(5,30)}$=189.112, *p<0.001*). All the concentrations (except the lower) killed the termites within an interval of 1 h.

Proportion of survival of *A. evuncifer*

The proportion of survival showed the way the death occurred amid termites after the contact with essential oils and controls. Like the survival duration, all the tested essential oils (at any concentration) affected the survival proportion. In both negative and positive controls, the survival proportion was consistent (1 for the two controls) during the first 6 h of bioassay (Figures 1, 2 and 3). From this value (1), it gradually decreased after 24 h from 0.99± 0.02 until the death of the last individuals at 936 and 960 h, respectively, for positive and negative controls. This suggests that solvent used to dissolve essential oils was totally evaporated and that it did not affect the termites.

For the essential oil of *C. elegans*, a quick decrease was observed for all the tested concentrations (Figure 1). In the lower concentration, the decrease started after 24 h until the 72 h with the death of the last termite. For higher concentrations (0.19 and 0.3 µl/cm²), the decrease started after the third hour of the bioassay and continued gradually until the sixth hour (0.93 ± 0.08 and 0.78 ± 0.19, respectively, for 0.19 and 0.3 µl/cm²). After the sixth hour, a quick and rapid straight death was observed between 24 and 48 h (time at which all the termites died).

For the fresh leaves of *R. reflexa*, the pattern was different (Figure 2). The lower dose (0.1 µl/cm²) of this essential oil decreased the survival proportion gradually similar to the controls until 576 h from the start of the bioassay. Conversely, no survival proportion was observed in the higher doses (0.19 and 0.3 µl/cm²) because all the termites were killed within the interval of 1 h. Similar pattern was observed for the essential oil of the dry leaves of *R. reflexa* (Figure 3). For this essential oil, the survival proportion was null 1 h after the starting of bioassay for all the tested doses except the lower one. It appeared that for all the tested essential oils, termites were gradually killed with the increasing of contact time with lower dose. However, higher doses straightly killed all the termites between 1 and 24 h.

Life expectancy of *A. evuncifer*

Life expectancy showed how long termites could survive after their exposition to different doses of tested essential oil samples. All the tested essential oils affected the life expectancy of *A. evuncifer* (Figure 4). The two way ANOVA showed that the life expectancy not only varied according to the essential oils ($F_{(2,105)}$=16.034, *p<0.001*) and the doses ($F_{(5,105)}$=622.107, *p<0.001*), but that there was an interaction between the essential oils and the doses ($F_{(10,105)}$=17.883, *p<0.001*). Among the essential oils, no significant difference was found between the global life expectancy of termites in contact with essential

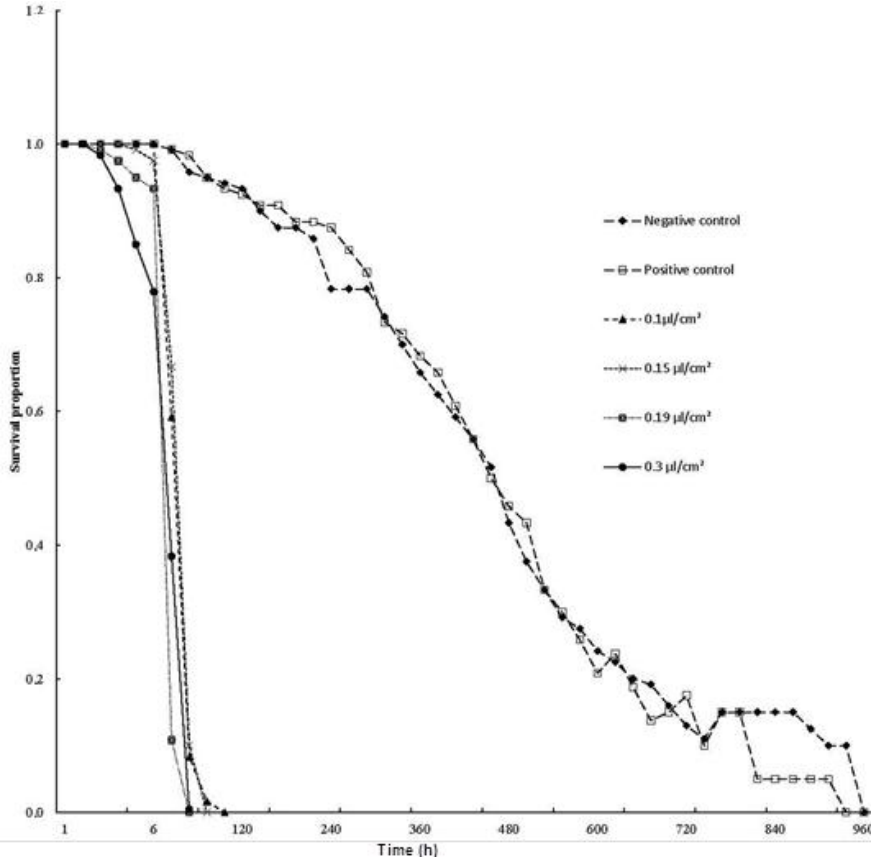

Figure 1. Survival proportion of *A. evuncifer* in contact with essential oil of *C. elegans*.
Source: Author

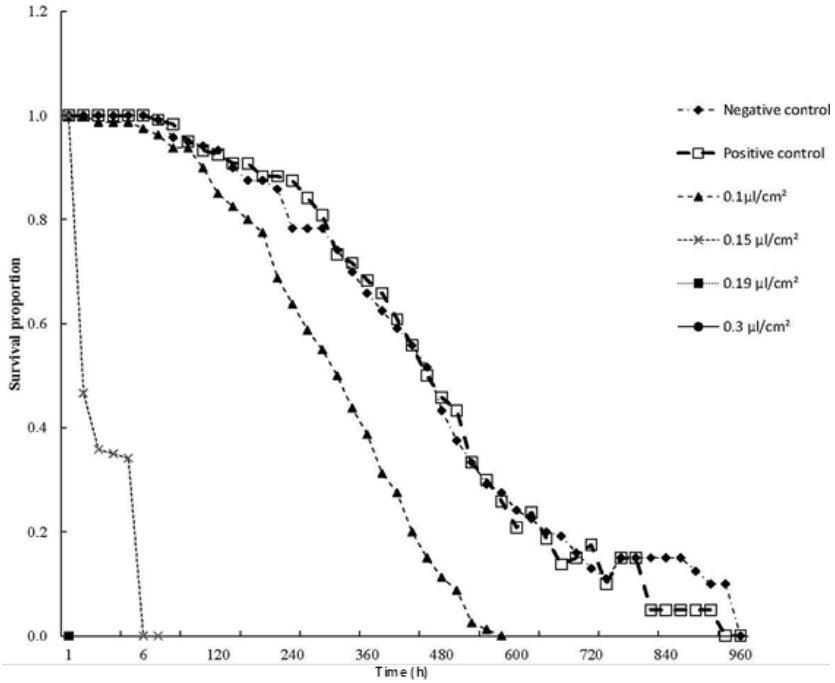

Figure 2. Survival proportion of *A. evuncifer* in contact with the essential oil of fresh leaves of *R. reflexa*.
Source: Author

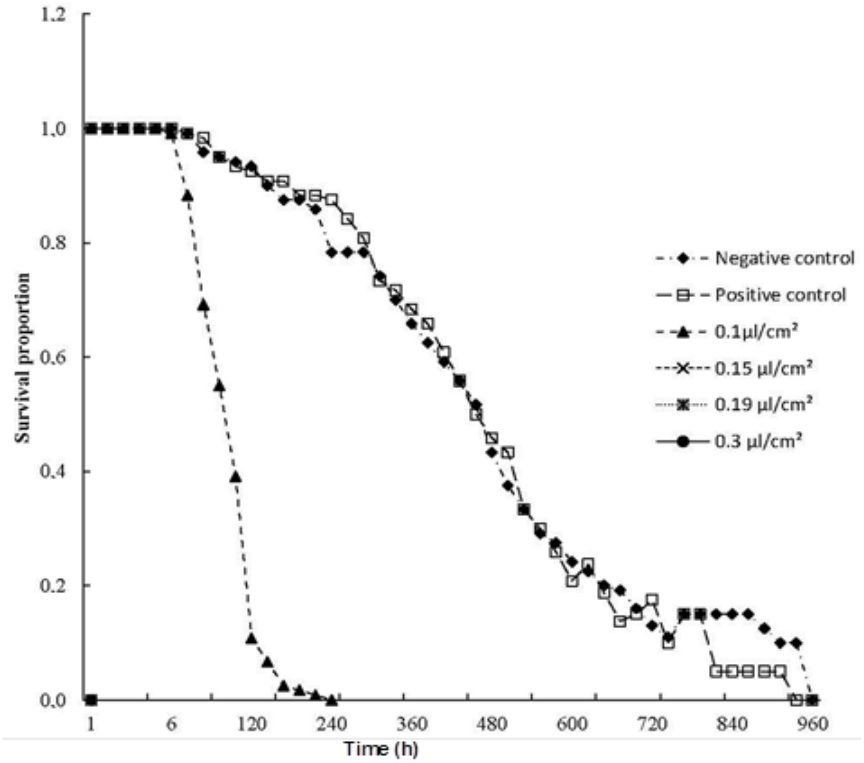

Figure 3. Survival proportion of *A. evuncifer* in conatct with essential oil of dry leaves of *R. reflexa.*
Source: Author

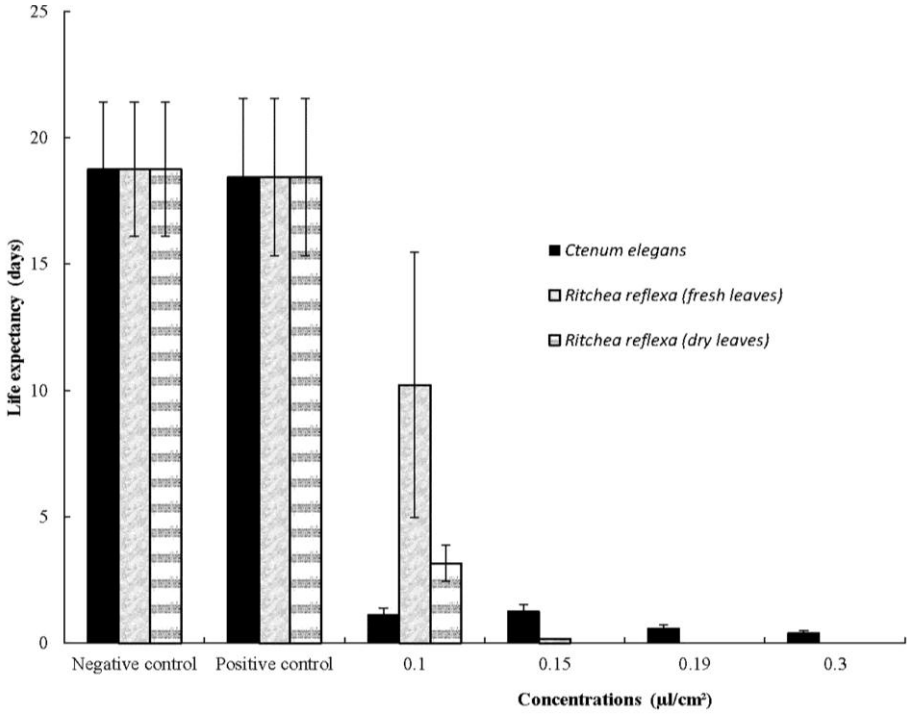

Figure 4. Life expectancy of *A. evuncifer* in contact of *C. elegans*, fresh and dry leaves of *R. reflexa.*
Source: Author

oils of *C. elegans* and the fresh leaves of *R. reflexa* (p=1, Bonferroni post hoc test). These essential oils seemed to have the same effect on the reduction of the life expectancy of *A. evuncifer*.

Conversely, a significant difference was found between the life expectancy of termites in contact with these two essential oils and that of the dry leaves of *R. reflexa* (*p<0.001* between *C. elegans* and p=0.001 between fresh leaves of *R. reflexa*). Thus, the essential oil of dry leaves of *R. reflexa* appeared to be the most efficient in the reduction of life expectancy of *A. evuncifer*. Also, the solvent have not affected the life expectancy as no difference was found between the two controls (p=1, Bonferroni post hoc test).

Within each essential oil, the effect on life expectancy varied according to the doses. For the essential oil of *C. elegans* ($F_{(5,30)}$=182.071, *p<0.001*), all the tested doses showed a significant difference between their respective life expectancy and that of control (*p<0.001*). However, no significant difference was found between life expectancy of termites from the tested doses (p=1, Bonferroni post hoc test). For this essential oil, the lower dose was just enough to cause a significant reduction of life expectancy (1.12±0.27 h) compared to the controls (18.76±2.66 and 18.45± 3.11 h, respectively, for negative and positive controls).

Different pattern was observed for the essential oil of fresh leaves of *R. reflexa*. For this essential oil, there was also a significant difference between the life expectancy of termite in contact of controls and the tested doses ($F_{(5,28)}$=207. 828, *p<0.001*). However, the lower dose was less efficient (*p<0.001*) than the other doses which similarly reduced the life expectancy of termite (p=1). The same pattern was observed for the essential oil of the dry leaves of *R. reflexa* ($F_{(5,35)}$=269.679, *p<0.001*). Its lower dose (even though significantly reduced the life expectancy, 3.14±0.72 h, compared to controls) was significantly less efficient (*p<0.001*) than the higher doses whose life expectancies were statistically similar (p=1 Bonferronii post hoc test).

DISCUSSION

Similar results of substantial amount loss of essential oils during a drying process of biomasses were reported (Rajeswara et al., 1992; Smigielski et al., 2011). According to the structures of the identified compounds in the case of *R. reflexa*, the three compounds were likely derived from the shikimate (phenylpropanoids) biosynthesis pathway (Başer and Buchbauer, 2010; Tisserand and Young, 2013; Wei et al., 2019). Benzyl cyanide has many known uses (Ackermann et al., 2000; Bien et al., 2000; Pollak et al., 2000), more as precursor of pharmaceutical products and was previously reported as a main constituent of essential oils of *Lepidium* species (Brassicaceae family) (Tellez et al., 2002; Saarivirta, 1973). Same origins were ascribed to benzyl isothiocyanate (Tellez et al., 2002; Saarivirta, 1973; Kermanshai et al., 2001) which was found as anthelmintic (Kermanshai et al., 2001). However, diisooctyl phthalate was mainly used as a minor plasticizer in plastic industry and has a limited toxicity (Carlson, 2010). Indeed, the content of Benzyl isothiocyanate dropped while that of Diisooctyl phthalate disappeared in oil sample of dried leaves of *R. reflexa*. Diisooctyl phthalate as ester was found to be volatile and so could be lost during drying process of leaves (Smigielski et al., 2011; Hassanpouraghdam et al., 2010). One could also suggest the conversion of such two compounds into their analogous Benzyl cyanide via a possible biosynthesis which might have continued during drying process of leaves (Smigielski et al., 2011). Even though it was reported that such phenylpropanoid derivatives were all degradation products of benzylglucosinolates (Tellez et al., 2002), no interconversion pathway among them had been indicated. As the yield of volatile oil of dried leaves was weak, the loss due to the volatility of such compounds was much more possible.

Elemol is widely used as fragrance ingredient in everyday commodities (Bhatia et al., 2008). The third main constituent is an epoxido-bicyclic α,β-unsaturated ester, and no-available literature concerning its bioactivity. The δ-cadinene and τ-muurolol, were also two known bioactive sesquiterpenes from essential oils (Zito et al., 2013; Buhagiar et al., 2015). So, the study presented evidence that mainly or exclusively phenylpropanoids derivatives could be isolated from the leaves of *R. reflexa* while full aerial part of *C. elegans* gave largely sesquiterpenes.

These results showed that the tested essential oils have affected *A. evuncifer*. The termiticidal effect against *A. evuncifer* of these essential oils might probably be caused by their (main) constituents (Benzyl cyanide, Benzyl isothiocyanate, and Diisooctyl phthalate (for *R. reflexa*), α-Cadinol, Elemol, Methyl (2Z)-3-(2,2,6-trimethyl-5-oxo-7-oxabicyclo[4.1.0]hept-1-yl)-2-propenoate, δ-Cadinene, and τ-Muurolol (for *C*. *elegans*). Although no activity was observed when isolated benzylcyanide of *Lepidium meyenii* essential oil was tested against the subterranean *Coptotermes formosanus* (Tellez et al., 2002), here the two oil formulations from *R. reflexa* showed that samples from dry leaves were more active against *A. evuncifer*. Indeed the termiticidal effect of many of these compounds or their analogous was reported by several studies (Tellez et al., 2002; Sakasegawa et al., 2003; Roszaini et al., 2013). Gupta et al. (2011) evaluated the potential of six plant-derived essential oils against *Odontotermes obesus*. They found that although all the tested oils exhibited termiticidal activity, those with phenolic moieties were most effectives. Pandey et al. (2012) found strong antitermitic activity against *Odontotermes assamensis* from several plant essential oils among which the

essential oil of *Cymbopogon citratus* (Lemongrass) exhibited the strongest termiticidal activity. The authors also indicated that the most active constituent of tested essential oils was phenolics.

The results of survival proportion of *A. evuncifer* in contact with the essential oils also proved the termiticidal effect of the tested essential oil samples. The gradual decrease of survival proportion observed in lower doses could be due to the repellency and antifeeding properties of essential oils as indicated by several authors (Zhu et al., 2001, 2003; Mishara and Dubey, 1994). However, the null survival proportion might be indubitably attributed to the toxicity of the tested essential oils. In most of the cases, the repellency and the toxicity of such essential oils might be a result of a combined effect of their main constituents. Thus, according to some authors Yang et al. (2004) and Park and Shin (2005), the efficiency of the activity of essential oil as antifeeding, insecticidal, repellent, and ovicidal is due to the synergistic or individual effect of its various constituents including alcohols, terpenoids, phenolics aldehydes as well as fatty acid derivatives.

The antifeeding, repellent and toxicity of essential oils might also explain the variation in the life expectancy of *A. evuncifer* (Zhu et al., 2001, 2003; Mishara and Dubey, 1994). Almost all the constituents of tested essential oils are known to exhibit microbial and antifungal activities (François et al., 2013; Jang et al., 2016; Saladino et al., 2017). Termites including *A. evuncifer* are also known to harbor several bacteria (*Bacillus subtilis*, *Bacillus cereus*, *Micrococcus luteus*, *Streptococcus* spp., and *Serratia marcescens*) in their hindgut (Femi-Ola and Aderibigbe, 2009). Termites gut microbiota is involved in the digestion of lignocelluloses (Mikaelyan et al., 2015) but also in nitrogen fixation (Benemann, 1973; Breznak et al., 1973) and its recycling (Brune and Ohkuma, 2010) which is very crucial for termites as their diet is very limited in nitrogen. Thus, the different constituents of essential oil, by targeting and killing termite microbiota seriously affect the termites feeding because they will not be able to efficiently digest the consumed lignocellulosic matters. These termites are thus condemned to die slowly or straightly according to the concentration of essential oil.

Conclusion

R. reflexa yielded 0.07% oil content on average with absolute presence (≥ 99%) of phenylpropanoid derivatives. *C. elegans* gave low oil content (0.006%) with a prevalence (58.6%) of oxygenated sesquiterpenes, followed by hydrocarbon sesquiterpenes (24.55%). Considering the low oil constituents (about 3 for *R. reflexa* and 14 for *C. elegans*) and the high contents of some of such constituents, further study is envisaged for isolation of pure main constituents. Termiticidal assays of such oil samples evidenced a promise antitermite activity against *A. evuncifer* responsible for severe damage of plants and food crops. Indeed, all the tested formulations at any dose affected not only the survival duration and the life expectancy but also straightly killed *A. evuncifer*. The lower doses because of their slow and significant efficient effect on *A. evuncifer* could be regarded as potential candidates for baiting trial in the IPM strategy for termites' control. For this purpose, further studies need to be carried in order to find out the exact effect (repellency, anti-feeding or toxicity) of these essential oils on *A. evuncifer*.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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