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Effect of powder and extract of *Ritchiea reflexa* (Thonn.) Gilg et Benedict and *Ctenium elegans* Kunth on *Amitermes evuncifer* Blattodea, Termitinae

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Termites play key roles in the ecosystems functioning, especially in the recycling of organic matter. However, few are considered pests because they cause damages to crops and wooden structures. Long based on the use of synthetic pesticides, the control of pestiferous termites is now focused on environmental friendly means including the use of plant extracts. Here, powder, acetic and ethanolic extracts of *Ctenium elegans* and *Ritchiea reflexa* were tested against *Amitermes evuncifer*. The phytochemical study on extracts exhibited tannins and terpenoids in both species, and steroids only in the ethanolic extract of *R. reflexa*. Five concentrations of each formulation were used against two controls (negative and positive). Six replicates were made for each concentration using 25 (for extracts) and 30 (for powder) healthy termite workers in petri dishes. All concentrations reduced the survival duration and life expectancy of termites. In 1.83 ± 0.41 days, the higher concentration (6 mg/cm^2) of *R. reflexa* ethanolic extract killed all termites against 6.67 ± 1.63 , and 8.67 ± 0.52 days for the same concentration of acetic extract and powder, respectively. On the other hand, it took 11.17 ± 2.64 ; 5.5 ± 0.55 , and 5.33 ± 0.52 days, for powder, ethanolic and acetic extracts of *C. elegans* to kill all the tested termites, respectively. The results suggested the use of these formulations as a botanical insecticide in termite management.

Key words: *Amitermes evuncifer*, biopesticides, *Ctenium elegans*, environment protection, *Ritchiea reflexa*.

INTRODUCTION

Termites (Blattodea) constitute one of the main biotic components of tropical ecosystems. where they represent, along with earthworms and ants, true engineers of soil (Duran-Bautista et al., 2020). Together

with ants, they represent nearly 65% of the biomass of soil fauna and are considered as bio-indicators for degraded soil or soil under restoration progress (Duran-Bautista et al., 2020). Termites play a key role in the

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functioning of the forest ecosystem (Ashton et al., 2019) where they act as recycler of organic matter (Jouquet et al., 2014). They are able to decompose large amount of deadwood (Griffiths et al., 2019). Despite their key role, some termites cause considerable damages to wooden structures in tropical countries. About 10% of termites are known to be harmful to living plants and buildings (Logan et al. 1990). They attack dead wood, living trees (Anani Kotoklo et al., 2010), food crops (Anani Kotoklo, 2007), plantations and furniture and other wooden structures inside habitations. Among termite pests, *Amitermes evuncifer* Silvestri, is one of the most devastating species that causes serious damage not only to dead and living trees (Ndiaye and Han, 2006) but also to building furniture. *A. evuncifer* has been described as the most devastating on fruits of trees and also the most damaging on palm oil trees (Sane et al., 2016). The damage caused by termites is often huge and the costs of repairing are enormous. Their control has long been based on the use of chemical insecticides such as organochlorines, organophosphates, and carbamates. These synthetic pesticides have adverse effects on human health and environment (Lemaire et al., 2004). For all these reasons, it becomes necessary to find reliable and environmentally friendly alternative methods for termite's management. Actually, the use of plant extracts and powders is attracting more attention. Indeed, several plants extracts or plant powders have been tested for their termiticidal properties. Addisu et al. (2014) tested five plants extract (including *Azadirachta indica*, *Jatropha curcas*, *Maesa lanceolata*, *Chenopodium ambrosoids* and *Vernonia hymenolepis*) against *Macrotermes* spp and found that all tested botanical extracts were efficient. Similar results were reached by Kasseney et al. (2016) with the extracts of three plants (including: *Cissus quadrangularis*, *Pennisetum purpureum* and *Vetiveria zizanioides*) against *Macrotermes subhyalinus* and *Trinervitermes geminatus*. The use of plant extracts and powders as an alternative for the control of insect pests is part of the environmentally friendly alternative methods used in agriculture. As part of our efforts to contribute to pest's management, two aromatic plants of the Togolese flora (*Ritchiea reflexa* and *Ctenium elegans*) have caught our attention. Indeed, the two plants are traditionally used in conventional medicine, as well as in the breeding and construction domains (Eyele, 1997; Sourabie et al., 1995). Moreover, the only study carried out on the two plants was related to their essential oils' chemical compositions. The study showed mainly the presence of the terpenoid compounds (monoterpenes and sesquiterpenes) and phenylpropanoids (Simalou, 2007), which family of compounds are all known as biologically active against insects and fungi (Tellez et al., 2002; Sakasegawa et al., 2003; Roszaini et al., 2013). To the best of our knowledge, the phytochemical screening and the termiticidal activity of *R. reflexa* and *C. elegans* extracts have not been previously reported and constitute

the aim of this paper. Thus, in this study, we evaluated the antitermite activity of six formulations including four extracts and two powders of *R. reflexa* and *C. elegans* against *A. evuncifer* in order to find out the good formulations for termite pests management.

MATERIALS AND METHODS

Plant material

Two aromatic plants from the Togolese flora, *R. reflexa* (Capparaceae) and *C. elegans* (Poaceae) were used for this study. The leaves of *R. reflexa* were collected in the locality of Zanguera (6°16'21.59292"N; 1°6'49.15152"E) and the leafy stems of *C. elegans* in the village of Danyi Koudzragan (7°8'29.1768"N; 0°37'30.21996"E). The two plants were morphologically identified by the Botanical Laboratory of the University of Lomé (Togo) and a lot number was assigned, TOGO 15508 (*R. reflexa*) and TOGO 15509 (*C. elegans*). The harvested leaves and stems were dried and crushed and the crushed material was kept in a cool place.

Preparation of *R. reflexa* and *C. elegans* extracts

Harvested leaves of *R. reflexa* were dried for two (2) weeks at room temperature and crushed like the leafy stems of *C. elegans*. 100 g of obtained powders for each plant were soaked separately in both 100 mL of acetone or ethanol and the mixtures were put under intermittent manual agitation during 72 h. The macerate of each solvent was evaporated by a rotary evaporator and the obtained extracts collected and stored inside a refrigerator (5 to 10°C).

Determination of chemical groups on extracts

Phytochemical screening and dosage tests were carried out on the two plant extracts in order to determine their main chemical groups. All methodology followed Chaouche et al. (2011). Flavonoids were detected by adding to 0.5 mL of each extract, ten drops of hydrochloric acid and few milligrams of magnesium turnings. After three minutes of incubation, the pinkish-red or yellow color indicated the presence of flavonoids. Similarly, the presence of tannins was detected by the changed coloration in the extracts when adding ferric chloride to 1% in 1 mL of extract and after a few minutes of incubation. Alkaloids were detected by adding 2 mL of hydrochloric acid (1%) into 1 mL of extract. The formation of a yellowish-white precipitate under Mayer's reagent and orange-red or brown under Bouchardat's reagent indicates the presence of alkaloids. Saponosides were also detected by adding 5 mL of distilled water to 2 mL of extract. The result was positive if the height of the foam was greater than 1 cm after 15 min of shaking. To determine steroids and terpenoids, 0.5 mL of chloroform and 0.7 mL of a sulfuric acid solution were added to 1 mL of extract. The green-blue color revealed the presence of steroidal heterosides and the green-violet color revealed the presence of terpene heterosides. Reducing compounds were detected by adding 2 mL of fehling liqueur to 2 mL of extract. After heating in the bath for 8 min, the formation of the brick-red precipitate indicated the presence of reducing compounds. For the detection of cardiotoxic glycosides, 2 mL of chloroform was added to 1 mL of extract, and the appearance of a reddish-brown coloration after the addition of H₂SO₄ indicated the presence of cardiac glycosides

Insects

A. evuncifer workers were used for the biological tests. Termites

were collected in the botanical garden of "Université de Lomé", Togo (06°30'14.9"N; 001°20'08.1"E) and acclimatized under laboratory conditions (28°C, 80% relative humidity) in total darkness (12:12H DD), 24 h before the start of the tests.

Bioassays test

Biological tests were performed by contact with extracts (acetic and ethanolic) and powders of *R. reflexa* and *C. elegans* following the methodology proposed by Raina et al. (2012).

Six formulations, powders (2), acetic (2) and ethanolic (2) extracts were used in this work. Five different concentrations of each formulation were compared with two controls (A and B). The control A consisted of a washer of filter paper placed inside a 9 cm diameter petri dish. Also, the control B consists of the same device as the control A except that the filter paper was impregnated with the extraction solvent (acetone or ethanol) and left in the open air for 24 h to remove the solvent. Control B was used to ensure that the solvent used for concentrations preparation had completely evaporated from the filter papers and therefore had no direct or indirect effect on the bioassays.

A total of six replicates were done for each tested concentration as well as for controls. Each concentration of powder was placed in a plastic petri dish with filter paper lined at the bottom. The powder was then evenly distributed over the entire surface of the washer of filter paper inside the petri dish. The respective masses of the concentrations of each extract were firstly dissolved in 5 mL of extraction solvent directly on the filter paper initially placed at the bottom of the glass petri dish (9 cm diameter). The whole was left in the open air for 24 h to remove the solvent. The different concentrations used were: 0.5, 1, 2, 4, and 6 mg/cm².

The workers of *A. evuncifer* were sorted and introduced in petri dishes whose bottom was lined with filter paper slightly soaked in distilled water. They were left for observation for three hours before being put in contact with the extracts. Twenty-five (25) workers were exposed to each concentration of extracts (acetic and ethanolic) of each tested plant. For plant powders, thirty (30) workers were also exposed to each of the five tested concentration. The petri dishes containing the termites were kept under laboratory conditions (28°C and 78% relative humidity) in total darkness (12:12 h DD). Termites were checked every six hours. At each checking time, dead and living individuals were counted and the dead were removed from the petri dishes. Verification was carried out after 6 h and after each 24 h until the death of the last individual.

Survival duration

Survival duration is the time that an individual termite spends in contact with the tested plant material before dying (Krebs 1999). It was estimated in days.

$$D_s = t_x - t_0$$

D_s : survival duration.

t_x : time spent by the last termite individual before its death.

t_0 : initial contact time of termites with different concentrations.

Life expectancy of termites

This is an estimate of the number of days an individual termite brought into contact with the extract is expected to reach before dying. Life expectancy was calculated using Ecological

Methodology 7.3 software (Krebs, 1999).

Statistical analysis

The two way analysis of variance (two-ways ANOVA) was used to check the difference between the survival durations and life expectancy of termites with the Bonferroni post hoc test. Then, the one way analysis of variance was used to check the difference between the survival duration and life expectancy of termites within the concentrations of each extract. These analyses were performed using SPSS 20 at the 5% probability threshold.

RESULTS

Phytochemical screening

The results obtained show the presence of tannins and terpenoids in the acetic and ethanolic extracts of *R. reflexa* and *C. elegans*. Only the ethanolic extract of *R. reflexa* revealed steroids (Table 1).

Effect of powder and extracts of *C. elegans* on survival duration of *A. evuncifer*

The powder and the two extracts of *C. elegans* affected the survival duration of *A. evuncifer* (Table 2). The analysis showed that there was a significant difference not only between the formulations ($F_{(2, 120)} = 42.925$, $P < 0.001$), but also between the concentrations ($F_{(6, 120)} = 89.858$, $P < 0.001$). However, no interaction was found between the treatments and the concentrations ($F_{(11, 120)} = 1.59$, $P < 0.113$).

Multiple comparison tests (Bonferroni post hoc test) showed that acetic and ethanolic extracts of *C. elegans* were significantly more effective than its powder in reducing the survival duration of *A. evuncifer* ($P < 0.001$). However, no significant difference was found between the two extracts ($p = 1$ Bonferroni post hoc test). To determine the most effective concentrations, the analysis was carried out within each formulation.

Thus, for the powder of *C. elegans*, all concentrations significantly reduced the survival duration of termites. However, no significant difference was observed between concentrations.

The acetic extract also significantly reduced the survival duration of termites. The reduction in survival duration was significant between concentrations 0.5 and 1 mg/cm² (Table 1). Concentrations 1 to 6 mg/cm² were not different.

The same pattern was observed for the ethanolic extract. Here all concentrations significantly reduced termite survival compared to the two controls (negative and positive). Between concentrations 0.5 and 1 mg/cm², the reduction of termite survival duration was concentration-dependent. Here also, concentrations 1 to 6 mg/cm² were not different.

Table 1. Results of the phytochemical screening of extracts.

| Extract | <i>Ritchiea reflexa</i> | | <i>Ctenium elegans</i> | |
|------------|-------------------------|--------------------|------------------------|--------------------|
| | Acetonic extract | Ethanollic extract | Acetonic extract | Ethanollic extract |
| Tannins | + | + | + | + |
| Terpenoids | + | + | + | + |
| Steroids | - | + | - | - |

+: presence; -: absence.

Table 2. Survival duration of termites in contact with the powder, acetonic and ethanollic extracts of *Ctenium elegans*.

| Formulation (mg/cm ²) | Survival duration (days) | | |
|-----------------------------------|---|---|---|
| | Powder | Acetonic extract | Ethanollic extract |
| Control A | 34±2.45 ^a | 28.67±2.88 ^a | 28.67±2.88 ^a |
| Control B | | 24.5±2.26 ^b | 26±2.19 ^a |
| 0.5 | 20.33±8.45 ^b | 14.17±1.47 ^c | 15.17±2.14 ^b |
| 1 | 21.5±11.59 ^{ab} | 6.67±1.51 ^d | 6.17±0.41 ^c |
| 2 | 14.50±8.87 ^b | 6 ^d | 6 ^c |
| 4 | 11.83±2.40 ^b | 4.5±0.55 ^d | 6.5±0.55 ^c |
| 6 | 11.17±2.64 ^b | 5.33±0.52 ^d | 5.5±0.55 ^c |
| | F _(5,35) =8.690; P<0.001 Bonferroni post hoc test | F _(6, 35) = 228.294. P<0.001 Bonferroni post hoc test | F _(6, 35) = 232.844. 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).

Effect of powder and extracts of *Ritchiea reflexa* on *A. evuncifer* survival duration

The results showed a significant difference between the three formulations of *R. reflexa* ($F_{(2,120)} = 71.209$, $P<0.001$) and also between the concentrations ($F_{(6,120)} = 191.516$, $P<0.001$). Analysis also showed a significant interaction between formulations and concentrations ($F_{(11,120)} = 2.174$, $p = 0.002$).

For the powder *R. reflexa*, reduction of survival duration was very significant. All concentrations of this formulation caused a significant reduction of *A. evuncifer* survival in the duration compared to controls. However, the concentrations of 4 and 6 mg/cm² were equally the most effective compared to controls and lower concentrations (Table 3).

The acetonic extract of *R. reflexa* also affected the survival duration of termites. All concentrations caused a significant reduction of the survival duration of survival of termites compared to the two controls. However, the three higher concentrations (2, 4 and 6 mg/cm²) were very equally efficient (Table 3).

The same pattern was observed for the ethanollic extract of *R. reflexa*. Thus, all concentrations significantly reduced the survival duration of termites compared to the controls that showed no significant difference between them. Here also, the three higher concentrations (2, 4 and 6 mg/cm²) were equally the most efficient (Table 3).

Effect of powders and extracts of *C. elegans* and *R. reflexa* on the life expectancy of *A. evuncifer*

Powders of *C. elegans* and *R. reflexa*

The powder formulation of both plants significantly affected termite life expectancy (Figure 1) ($F_{(5,30)}=45.654$, $P<0.001$ for *C. elegans* and $F_{(5,30)}=26.232$, $P<0.001$ for *R. reflexa*). For both *C. elegans* and *R. reflexa*, all concentrations significantly ($P<0.001$) reduced termite life expectancy compared to control (22.66±3.55 days). Moreover, the reduction in life expectancy was not concentration-dependent for this formulation (powder) because no significant difference ($0.162 \leq p \leq 1$) was found between the values of life expectancy of different concentrations (Figure 1). Unlike *C. elegans* powder, the reduction of life expectancy caused by *R. reflexa* powder was concentration-dependent. Concentrations of 4 and 6 mg/cm² were the most effective (8.73±1.39 and 7.43±0.81 days respectively).

Acetonic extracts of *C. elegans* and *R. reflexa*

Acetonic extracts from both plants also caused a significant reduction in termite life expectancy ($F_{(6,35)}=178.966$, $P<0.001$ for *C. elgans* and $F_{(6,35)}=41.708$, $P<0.001$ for *R. reflexa*) compared to both control A and B

Table 3. Survival duration of termites in contact with the powder, acetonc and ethanolic extracts of *Ritchea reflexa*.

| Formulation (mg/cm ²) | Survival duration (days) | | |
|-----------------------------------|--|--|---|
| | Powder | Acetonc extract | Ethanolic extract |
| Control A | 34±2.45 ^a | 28.67±2.88 ^a | 28.67±2.88 ^a |
| Control B | | 24.5±2.26 ^a | 26±2.19 ^a |
| 0.5 | 20.5±3.39 ^b | 16±2.1 ^b | 12.83±5.34 ^b |
| 1 | 19.83±2.48 ^b | 15.17±4.67 ^b | 11.83±4.36 ^b |
| 2 | 17.33±1.86 ^b | 8.33±2.66 ^c | 4.83±2.4 ^c |
| 4 | 11.50±2.17 ^c | 7.17±2.4 ^c | 4.17±1.72 ^c |
| 6 | 8.67±0.52 ^c | 6.67±1.63 ^c | 1.83±0.41 ^c |
| | F _(5,35) =88.142. P<0.001 Bonferroni post hoc test | F _(6, 35) = 57.618. P<0.001 Bonferroni post hoc test | F _(6, 35) = 69.01. 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).

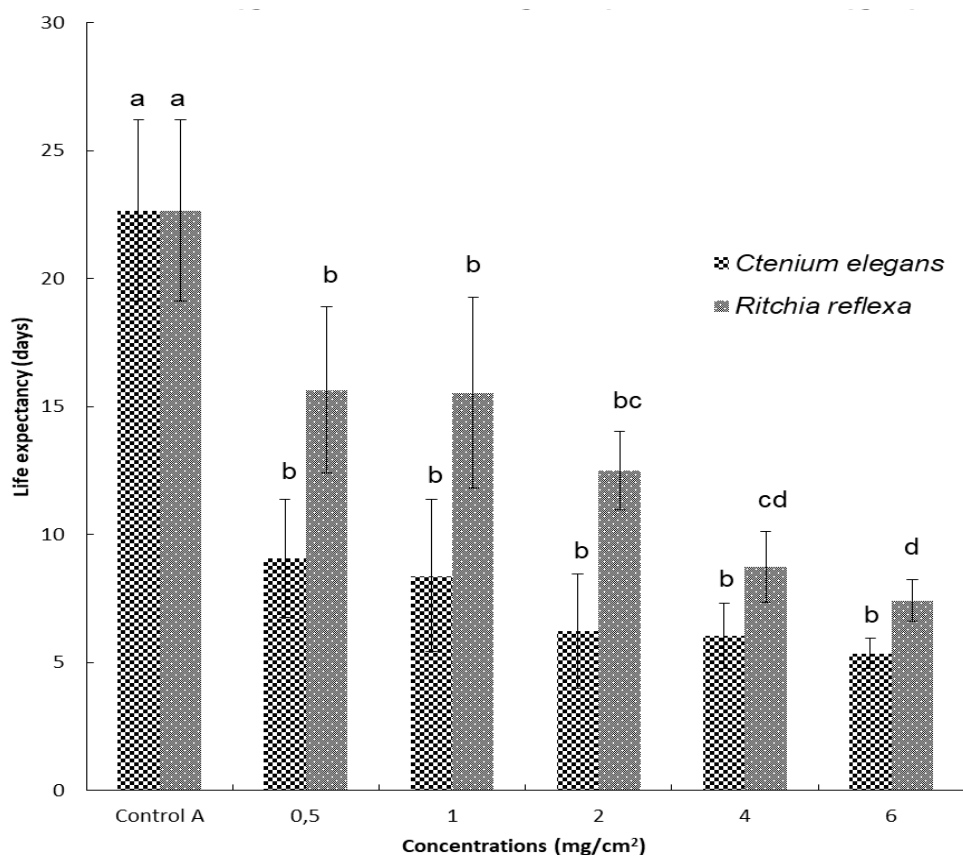


Figure 1. Life expectancy of *Amitermes evuncifer* in contact with *Ctenium elegans* and *Ritchea reflexa* powders. Life expectancies from concentrations of the same plant following by the same letter are not different.

(16.58±2.17 and 17.11±1.89 days respectively).

For *C. elegans*, the 0.5 mg/cm² concentration was the least efficient while no difference was found between the life expectancies of higher concentrations (0.651≤P≤1).

For *R. reflexa* acetonc extract, the smallest reductions of life expectancy were recorded in the 0.5 and 1 mg/cm²

concentrations (11.59±0.7 and 11.02±0.61 days respectively). No difference was found between the life expectancies recorded in the higher concentrations (5.98±2.08; 4.93±1.26 and 4.92±2.09 days for concentrations 2, 4, and 6 mg/cm² respectively) (Figure 2).

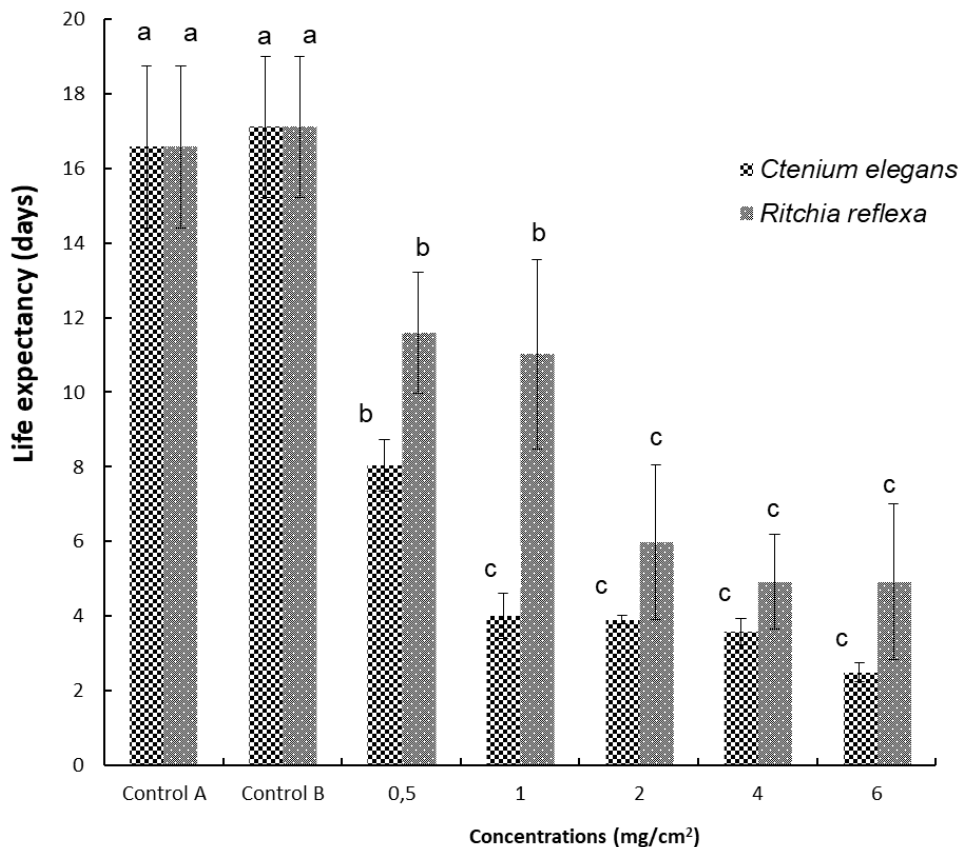


Figure 2. Life expectancy of *Amitermes evuncifer* in contact with acetonic extracts of *Ctenium elegans* and *Ritchia reflexa*. Life expectancies from concentrations of the same plant following by the same letter are not different.

Ethanollic extracts of *C. elegans* and *R. reflexa*

The ethanollic extract of both plants also affected the life expectancy of termites ($F_{(6,35)}=85.904$ $P<0.001$ for *C. elegans* and $F_{(6,35)}=51.406$ $P<0.001$ for *R. reflexa*). For *C. elegans*, although all concentrations caused significant reduction of life expectancy ($P<0.001$), the 0.5 mg/cm² concentration was the least effective (9.09 ± 1.72 days) compared to the other concentrations. In contrast, for *R. reflexa*, the 0.5 and 1 mg/cm² concentrations were the least effective (9.1 ± 2.96 and 8.58 ± 3.14 days respectively) (Figure 3). Concentrations 2, 4 and 6 mg/cm² of the ethanollic extract of this plant were very effective in reducing the life expectancy of termites (3.55 ± 1.27 , 2.57 ± 1.09 and 1.29 ± 0.4 days respectively).

DISCUSSION

The phytochemical study carried out on both species revealed the presence of tannins and terpenoids in their acetonic and ethanollic extracts. Steroids were only found in ethanollic extract of *R. reflexa*. To our knowledge, this is the first phytochemical tests realized on the two plants;

hence it is hard to stand a comparison. However, this phytochemical study represents an essential asset that will allow us to support the insecticidal properties of both plants. According to Nsambu et al. (2014), secondary metabolites constitute new potential sources of control of insect pests of crops or stored commodities. Tahiri et al. (2010) have shown that terpenoids have insecticidal, fungicidal, and repellent properties. Also, Zhang et al. (1990) have also shown that tannins are compounds that possess insecticidal, larvicidal, and repellent properties. Therefore, the existence of terpenoids, tannins, and steroids in the extracts of the two plants might be responsible for the presence of several properties in both plants.

Compared to control A, the control B showed no effect on termites, which proves that the solvents used to dilute the extracts, totally volatilized and could not influence the bioassays. On the other hand, all concentrations of the powders and extracts showed an effect against termites by significantly reducing the survival duration and life expectancy of termites. This justifies the existence of certain toxic substances present in the extracts that act on termites, such as tannins, terpenoids and steroids (Ahmad et al., 2019). Indeed, tannins are known to be

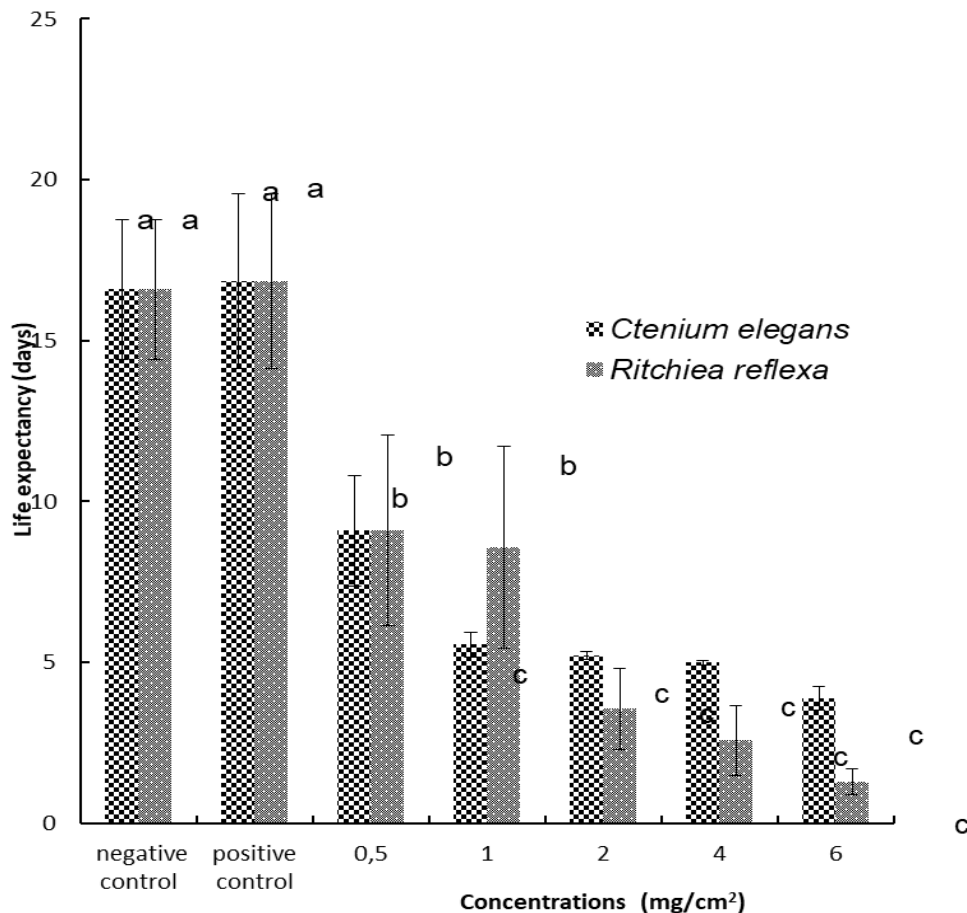


Figure 3. Life expectancy of *Amitermes evuncifer* in contact with ethanolic extracts of *Ctenium elegans* and *Ritchiea reflexa*. Life expectancies from concentrations of the same plant following by the same letter are not different.

one of the environmentally friendly compounds used in wood preservation against termites and other wood dwelling organisms (Verly Lopes et al., 2020). Yamaguchi et al. (2002) treated some woods with several tannins solutions and found contact lethality for *Coptotermes formosanus* (one of most pestiferous subterranean termite) in the contact toxicity bioassays. Krupal and Narasimhacharya (2017) tested extracts of four plant species against *Odontotermes obesus* and found that the most efficient extracts were those containing a higher quantity of tannins along with alkaloids, flavonoids and saponins. Like tannins, terpenoids are also reported, not only to be repellent but also toxic to termites (Sharma et al., 1994; Bezuneh et al., 2019). Termiticidal effect of terpenoids and steroids (extracted from *Prosopis juliflora*) against *Macrotermes* spp was related by Bezuneh et al. (2019). Steroids isolated from the extract of *Hopea mengerawan* were found to be efficient against *Cryptotermes cynocephalus* (Sugita et al., 2000). Thus, the tested formulations (powder and extract) own their termiticidal properties to these compounds.

Although both were efficient against termites, the powders and extracts of the two plant species did not show the same degree of efficiency. Overall, the extracts were more effective on *A. evuncifer* workers than the powders. According to Osbrink et al. (2001) and Osbrink & Lax, (2002), the insecticidal activity of plants varies according to the formulations and termite species. Similarly, according to Pettersen, (1984), the termiticidal properties of plants are mainly based on secondary metabolites or extractable organic materials such as extracts, waxes, alkaloids, fats, gums, resins, terpenes and essential oils.

For both plant species, the ethanol extracts showed a more effective insecticidal effect than the acetone one. Indeed, Teugwa et al. (2002) stated that the efficacy of a product would depend on the solvent used. The active substances against termites were thus more extracted by ethanol than acetone, and more soluble in ethanol than acetone. This suggestion was confirmed by the phytochemical screening of the ethanolic extract, which revealed that in addition to the tannins and terpenoids

that were present, there were also steroids that are otherwise absent in the acetone extracts.

Conclusion

The phytochemical screening performed on the extracts of both species showed the presence of terpenoids and tannins in the extracts. Moreover, steroids were found in the ethanolic extract of *R. reflexa*. Indeed, biological tests showed that all concentrations of the powders as well as the extracts of both plants showed an insecticidal effect against the termite species *A. evuncifer*. For the same concentration, all the extracts (acetonic and ethanolic) of the two plants reduced the survival duration and life expectancy more than the powders. This indicated that the extracts were more effective than the powders. Also, the ethanolic extract of *R. reflexa* reduced the life expectancy and survival duration of the termites more than the other extracts and was therefore more active. Therefore, the two plant species might potentially be considered as bio-insecticides in the management of *A. evuncifer* species.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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