

Chapter

Termiticidal Activity of *Senna occidentalis* and *Tithonia diversifolia* Extracts and Study of Their Toxicity on Mammals

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Abstract

The termiticidal activity of methanolic extracts of *Senna occidentalis* and *Tithonia diversifolia* leaves was determined on the crop pest termite, *Ancistrotermes cavithorax*. Both extracts were toxic to termite workers by contact and inhalation. The *T. diversifolia* extract (LC₅₀ of 29, 76 mg/l) was more toxic than the *S. occidentalis* extract (LC₅₀ of 84.90 mg/l). The two extracts are not repellent but anti-palatable. To recommend insecticidal plants in the culture medium requires the knowledge of their harmfulness to the environment and mammals. This and the toxicity of its two extracts were determined on female rats *Rattus norvegicus*. The LD 50 of these two extracts exceeded 5000 mg/kg of body mass. The extracts did not cause any renal or hepatic damage after 14 days. The use of these insecticidal plant extracts can therefore be recommended to farmers.

Keywords: toxicity, termites, mammals, *Tithonia diversifolia*, *Senna occidentalis*

1. Introduction

Termites are one of the greatest scourges in tropical agriculture and agroforestry [1, 2]. In Côte d'Ivoire, West Africa, they regularly attack and destroy food and industrial crops [3–6]. To control termite attacks and damage, several methods are used by producers. Among these techniques is the use of chemical inputs [7]. Excessive use of chemical inputs can adversely affect living organisms and their environment [8] with very high repair costs. In sub-Saharan Africa, the potential cost of treating pesticide-related illnesses between 2005 and 2020 is about US\$90 billion [9]. These problems with synthetic pesticides have led to the search for new termiticidal substances from plants that are environmentally and human health friendly [5, 10, 11]. Pesticidal plants are toxic to pests and generally less harmful to beneficial insects and the environment [12]. The present study set out to evaluate the insecticidal properties of two common plants in West Africa, *S. occidentalis* and *T. diversifolia*, on the crop pest termite, *Ancistrotermes cavithorax*. The toxicity of these two plants will also be evaluated on the rat *Rattus norvegicus*.

2. Materials and methods

2.1 Plant materials

Fresh leaves of *S. occidentalis* and *T. diversifolia* were collected for this study in May 2017 in the commune of Bingerville (Abidjan – Ivory Coast) (between 6 and 7 am). The harvested samples were cleaned, air-dried at room temperature, and protected from light for 2 weeks. They were then looted and well-preserved until use.

2.2 Animal material

The pesticide tests were conducted on adult workers of the termite *Ancistrotermes cavithorax* captured in underground nests in the commune of Bingerville (Abidjan – Ivory Coast).

The acute toxicity tests and the histopathology study were conducted on *R. norvegicus* rats of the Wistar strain from the animal house of the Ecole Normale Supérieure d'Abidjan (ENS). These rats are virgin, nulliparous, nonpregnant, 6–8 weeks old, and between 140 and 160 g. The rats were exposed to 5 days of acclimatization with free access to water and food *ad libitum*. The bedding was renewed twice a week.

2.3 Preparation of extracts

Extracts were prepared according to the method described by Kaushik and Vir [13]. Thus, 30 g of powder from the leaves of each plant was mixed in 1 l of 70% methanol. The resulting mixture was stirred for 48 h at room temperature (25°C) using a magnetic stirrer type IKAMAG RCT (Staufen, Germany). The mixture was filtered three times on Whatman paper (No. 4) and then using cotton. The collected solution was subjected to rotary evaporation with a rotavapor BUCHI R-100/32 to obtain the methanolic extract. This solution was then dried under vacuum.

2.4 Biological tests

2.4.1 Toxicity by contact

The test was carried out in Petri dishes 90 mm in diameter containing 3.5 g of soil moistened with 1 ml of distilled water [14]. Using a micropipette, 10, 20, 50, and 100 µl doses of each extract were deposited and mixed with the soil. After depositing, the dishes are air-dried for 1 h. Fifty adult workers of *A. cavithorax* termites were introduced into each of these Petri dishes. Each extract is tested at four doses 10, 20, 50 and 100 µl. Each dose was repeated six times. Each control box was treated with the solvent (70% methanol + distilled water). Dead workers were counted every 2 h. After 24 h, the count was carried out every day until all insects were dead. Percentage mortality (PM) is calculated as the ratio of the observed number of deaths to the total number of insects:

$$PM = \frac{\text{Observed mortality}}{\text{Total number of insects}} \times 100 \quad \text{Total number of insects} = 50/\text{box}. \quad (1)$$

LD50 is calculated on the basis of 24-h mortality.

2.4.2 Inhalation toxicity

The inhalation test was performed in a large Plexiglass® box of 180 × 20 × 70 mm height according to the method of Tahiri et al. [14]. Into each large box was introduced a Petri dish containing 3.5 g of soil previously moistened with 1 ml of distilled water. Fifty *A. cavithorax* workers are introduced into the Petri dishes, which are then closed with a mosquito net lid. Using a micropipette, the 100 µl dose is placed on 4 cm² Whatman® paper which is then transferred to the large dish. The device was then hermetically sealed. Each tested solution was repeated six times for all the tests. Each control box was treated with the corresponding solvent. Worker mortality was determined after 24 h of treatment.

2.4.3 Barrier soil test

The barrier soil test was conducted according to the method of Tahiri [11]. Nine plastic boxes (chambers) (diameter 5 × 3.5 cm high), closed with plastic covers, were used per arena. One introduction chamber containing sieved nest soil, four substrate chambers containing nest soil, and four food chambers, each containing two pieces of Whatman no. 4 paper of 4 cm² were used.

The chambers were connected to each other and to the introduction chamber located in the center of the arena by transparent tubes (7 cm length) (LABELL Ch/Fr), length 49 inches. In each trial, only one substrate chamber per arena was treated as barrier soil (50 g of sand moistened with 7.5 ml of the product) to prevent termites from reaching the food source. The other untreated substrate chambers contained 50 g of sand moistened with 7.5 ml of distilled water.

Three hundred *A. cavithorax* workers are weighed before being introduced into the arena through the introduction chamber. They remained isolated for 24 h in this chamber (clamped connecting tubes) to acclimatize before moving freely through all chambers of the arena. The arena is maintained in a room at 27°C. Three replicates with 300 termites were performed for each trial with the extracts and distillates of *S. occidentalis* and *T. diversifolia* and for each corresponding control.

After 10 days of continuous exposure to the product, surviving workers are counted in all arenas, and their total weight is calculated. The tunneling task and food consumption of workers were compared between treated and untreated insects.

2.4.4 Acute toxicity in female rats

The experiment was conducted according to the European OECD guideline 423 [15]. Nine female rats were divided into three batches of three rats (one control and two treated batch) and used for the experiments. The animals were deprived of food overnight. Prior to the experiment, the animals were provided free access to water. Control animals received ml/100 g body weight of distilled water. Due to the low toxicity of *S. occidentalis* [16] and *T. diversifolia* [17] leaves, the treated batch received a single dose of 2000 mg/kg body weight of the treatment *T. diversifolia* or *S. occidentalis*. Each animal received 1 ml/100 g body weight of either *T. diversifolia* or *S. occidentalis* extract. The change in general behavior or mortality of rats in each batch was monitored for 14 days. After this time, the animals were introduced into boxes containing cotton soaked in ether in order to anesthetize them. After anesthesia, the animals were sacrificed.

2.4.4.1 Relative mass of vital organs

At the end of the 14-day test, each animal was weighed and then sacrificed. The kidneys, liver, and heart were removed and weighed. Relative masses were calculated according to the following formula:

$$\text{Relative mass (\%)} = \frac{\text{Mass of the organ}}{\text{mass of the animal}} \times 100 \quad (2)$$

2.4.4.2 Hematologic and biochemical examinations

At the time of animal sacrifice, volumes of blood were collected in EDTA tubes and dry tubes for hematologic and biochemical analysis, respectively. The hematologic analysis was performed using a Sysmex XS-500i automated analyzer. The blood count consisted of determining the number of white blood cells (WBC), red blood cells (RBC), and blood platelets (PLT), the hematocrit (HCT) and hemoglobin (HGB), the mean blood volume (MCV), the mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC).

Biochemical analysis of blood was performed after centrifugation at 3000 rpm for 4 min. An aliquot of serum was collected in Eppendorf tubes. Urea, creatinine, blood glucose, cholesterol, alanine aminotransferase (ASAT), and aspartate aminotransferase (ALAT) were determined using a HITACHI 704 R (Japan) automated analyzer.

2.4.4.3 Histopathology of the liver, heart, and kidney

During the sacrifice, the liver, heart, and kidney of treated and control rats were removed, rinsed with 9‰ NaCl solution, and then fixed in 10% formalin. Flaps of each organ were arranged in cassettes and successively bathed in 80°, 90°, 96°, and 96° ethanol for dehydration. They were then cleared in toluene I and II for 1, 2, and 2 h and then cleared in a step for 2 h in the same reagent. The cassettes were removed from toluene, drained, and impregnated, respectively, in two liquid kerosene baths (I and II) for 2 and 3 h in an oven at 50°C. The cassettes were then cured in the open air and then in the freezer. Sections of 5 µm were made with a Leica RM 2125 RTS® microtome and then stained with hematoxylin and eosin (H&E). The sections were observed with an Olympus CKX41 microscope (Germany) connected to a computer equipped with Videomet software. Pictures of the different histological sections were taken.

2.5 Statistical analysis

The results were expressed as mean plus or minus standard deviation, compared by the ANOVA test and separated by the Newman–Keuls test at the 5% threshold. The statistical software used was Statistica 7.0.

3. Results

3.1 Toxic effect by contact of the extracts

The results of the contact toxicity test indicate that the methanolic extract of *S. occidentalis* causes 22.8% mortality after 24 h and 100% after 96 h at the concentration of 10 mg/l, while the control shows 2.4% mortality after 24 h and 100% after

144 h. Concentrations of 1, 2, and 5 mg/l cause less than 20% mortality after 24 h and 100% after 120 h. Termite mortality increased proportionally with concentration and exposure time. Fifty percent (50%) of the mortality of *A. cavithorax* workers was obtained before 48 h of treatment at the 10 mg/l concentration (**Figure 1**). The Kruskal–Wallis test indicated that the total methanolic extract of *S. occidentalis* caused a significantly different mean number of mortalities than the control at 24 h ($P < 0.05$), 48 h ($P < 0.05$) and 72 h ($P < 0.05$).

Percentage mortality as a function of time for the contact toxicity test of the methanolic extract of *Tithonia diversifolia* showed that the 10 mg/l concentration causes 30.8% mortality after 24 h and 100% after 120 h. Concentrations 1, 2, and 5 mg/l caused less than 15% mortality after 24 h and 100% after 120 h, while the control showed 1.2% mortality after 24 h and 100% after 144 h.

The mortality rate of termites increased proportionally with the concentration and time of exposure. Fifty percent (50%) of the mortality of *A. cavithorax* workers is obtained before 48 h of treatment with the 10 mg/l concentration (**Figure 2**). The Kruskal–Wallis test indicated that the extract of *T. diversifolia* tested caused a significantly different average number of mortality than those caused in the control in 24 h ($P < 0.05$), in 48 h ($P < 0.05$), in 72 h ($P < 0.05$) and in 96 h ($P < 0.05$).

3.2 Lethal concentrations of extracts

The Probit analysis performed on the basis of 24-h mortality, showed that the amount of the methanolic extract of *T. diversifolia* required to kill 50% of the tested termite population, is 297.657 μ l, or a lethal concentration 50 (LC50) of 29.76 mg/l. With the methanolic extract of *S. occidentalis*, the dose required to kill 50% of the termite population tested, is 849.068 μ l, or a lethal concentration 50 (LC50) of 84.90 mg/l of extract (**Table 1**).

3.3 Inhalation toxicity of extracts

After 24 h of inhalation, the mortality rates of 95.99% and 91.66% obtained with the methanolic extract of *T. diversifolia* and *S. occidentalis* leaves, respectively, were

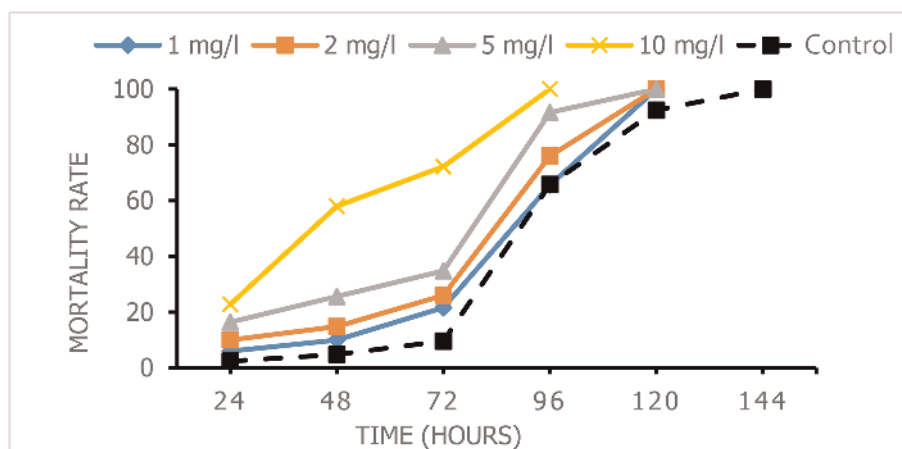


Figure 1.
 Variation in mortality of *Ancistrotermes cavithorax* after exposure to methanolic extract of *S. occidentalis*.

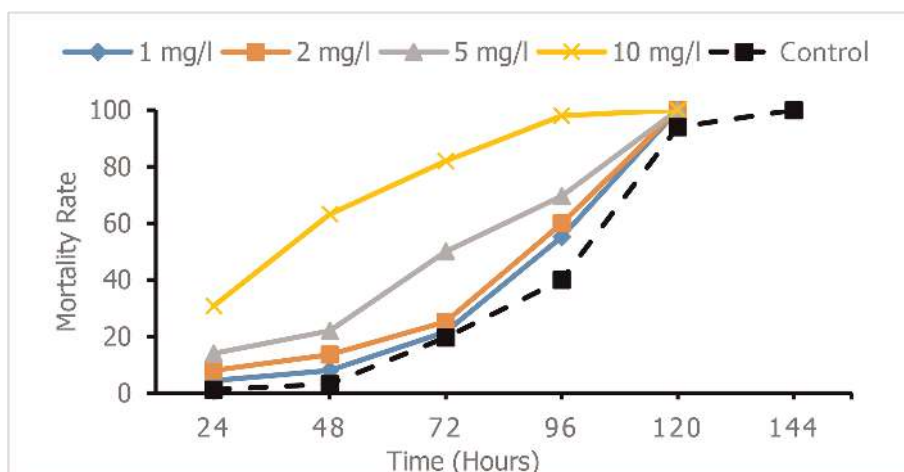


Figure 2.

Variation in mortality of *Ancistrotermes cavithorax* after exposure to methanolic extract of *Tithonia diversifolia*.

Products	LC50 (mg/l)	Boundary below 95% (mg/l)	Borne above 95% (mg/l)
<i>T. diversifolia</i> extract	29.76	19.87	55.90
<i>S. occidentalis</i> extract	84.90	37.57	426.22

Table 1.

Lethal concentration 50 (LC50) of the total methanolic extract of *S. occidentalis* and *Tithonia diversifolia*.

higher than that obtained in the control. Statistical analysis indicated that the mortality rate obtained with the total methanolic extract of *T. diversifolia* and *S. occidentalis* was significantly higher ($P < 0.05$) than that of the control (Figure 3).

3.4 Toxic effect by barrier soil of extracts

In the test and control arenas, after 10 days of continuous exposure of 300 workers to moistened soil, the mortality rates of $88.89 \pm 3.37\%$ and $85.33 \pm 9.61\%$, respectively, in the tests with *T. diversifolia* extracts with and *S. occidentalis* were significantly higher than that obtained in the controls ($P < 0.05$) (Table 2). Mortality was unevenly distributed across the four substrate and food chambers in both trials and controls. In the test arenas, the substrate chamber treated with methanolic extracts of both extracts is visited by termites. However, no paper (food bait) was located after the treated substrate chamber was consumed. In contrast, papers in the other untreated substrate chambers of the arena were consumed, but with significantly lower consumption ($P < 0.05$) than in the control areas (Table 3).

3.5 Mortality and symptoms in female rats

Single-dose oral administration of 2000 mg/kg body weight of methanolic extract of *T. diversifolia* and *S. occidentalis* to female rats resulted in no deaths after 14 days. Furthermore, observation of the animals after 30 min, 4 and 24 h, and then regularly

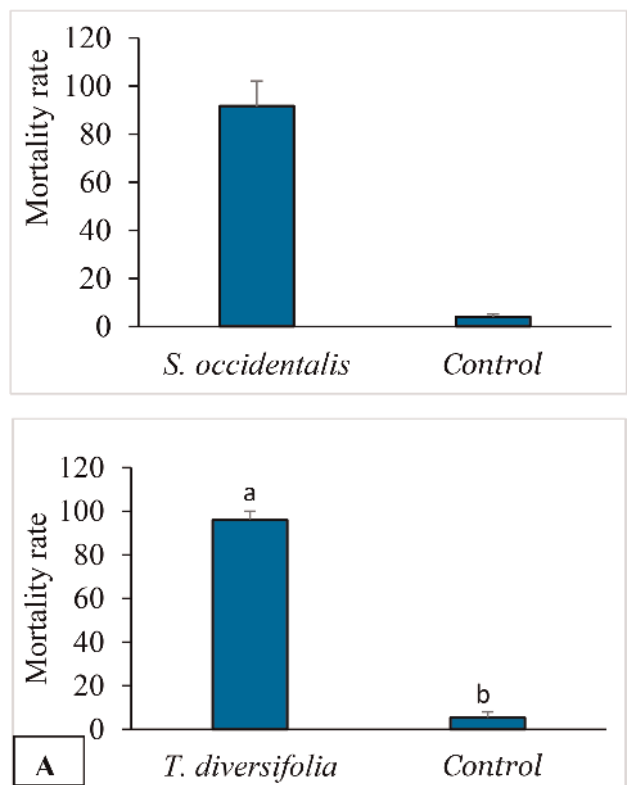


Figure 3.
Distribution of mortality of *Ancistrotermes cavithorax* workers during the inhalation test. A: Inhalation test with methanolic extract of *Tithonia diversifolia*. B: Inhalation test with methanolic extract of *S. occidentalis*.

Soil moistening	Average mortality (%) $\pm$ standard deviation
Extract of <i>S. occidentalis</i>	85.33 $\pm$ 9.61 a
Extract of <i>Tithonia diversifolia</i>	88.89 $\pm$ 3.37 a
Distilled water	26.0 $\pm$ 1.85 c

Values followed by the same letters in the same column are not significantly different at the 5% threshold (Kruskal–Wallis). N = 300 workers/test; paper area = $2 \times 4 \text{ cm}^2/\text{room}$.

Table 2.
Average mortality rate of *Ancistrotermes cavithorax* workers after 10 days during the barrier soil test.

every 24 h for 14 days showed no signs of toxicity (salivation, drowsiness, morbidity, and coma). The animals showed signs of well-being (active movement, normal intake of food and water) compared to controls (**Table 4**).

3.6 Effects of extracts on relative organ mass

There was no significant change in the relative mass of the kidneys, liver, and heart of treated rats compared with the relative mass of the organs of control rats (ANOVA, $P > 0.05$) (**Table 5**).

Soil moistening	Cumulative consumption (mm ²) ± standard deviation
Extract of <i>S. occidentalis</i>	08.04 ± 1.82 a
Extract of <i>Tithonia diversifolia</i>	06.09 ± 1.5 a
Distilled water	175.92 ± 8.46 c

Values followed by the same letters in the same column are not significantly different at the 5% threshold (Kruskal–Wallis). N = 300 workers/test; paper area = 2 × 4 cm²/room.

Table 3.
Cumulative consumption of *Ancistrotermes cavithorax* workers after 10 days during the barrier soil test.

Rats	Doses (mg/kg)	Number of dead rats	Symptoms
Lot 1 (Control, n = 3)	0	0	—
Lot 2 (EMSO, n = 3)	2000	0	—
Lot 3 (EMTD, n = 3)	2000	0	—

EMSO, Methanolic extract of *S. occidentalis*; EMTD, Methanolic extract of *T. diversifolia*.

Table 4.
Acute toxicity to female rats of methanolic extracts *Tithonia diversifolia* and *S. occidentalis*.

Parameters	Lot 1 control	Lot 2 <i>S. occidentalis</i>	Lot 2 <i>T. diversifolia</i>
Kidneys	0.256 ± 0.005 a	0.268 ± 0.006 a	0.256 ± 0.005 a
Livers	3620 ± 0.300 a	3920 ± 0.360 a	3780 ± 0.270 a
Heart	0.351 ± 0.013 a	0.335 ± 0.003 a	0.326 ± 0.001 a

Values assigned to the letter a in the same row do not differ significantly ($P > 0.05$).

Table 5.
Effects of total methanolic extract of *Tithonia diversifolia* and *S. occidentalis* on the relative weight of organs harvested from rats.

3.7 Effects of extracts on biochemical and hematologic parameters

The urea level of rats treated with methanolic extract of *T. diversifolia* (0.37 ± 0.015 mmol/l) was not significantly different (ANOVA, $P > 0.05$) from the control (0.37 ± 0.026 mmol/l). However, the urea level of rats treated with methanolic extract of *S. occidentalis* (0.27 ± 0.023 mmol/l) was significantly lower (ANOVA; $P < 0.05$) than the control. Aspartate aminotransferase (ASAT) levels in the blood of rats treated with methanolic extracts of *T. diversifolia* (134.7 ± 1.76 IU) and *S. occidentalis* (131.3 ± 1.85 IU) were significantly lower (ANOVA, $P < 0.01$) than that of the control (161.5 ± 1.964 IU). Blood glucose, total cholesterol, and alanine aminotransferase (ALAT) levels observed in total extract-treated rats were not significantly different (ANOVA; $P > 0.05$) from the control (**Table 6**). In addition, hematologic analysis showed no significant change (ANOVA; $P > 0.05$) in the blood parameters of the methanolic extract-treated rats (**Table 7**).

Parameters	Control (distilled water)	<i>T. diversifolia</i> (2000 mg/kg bw)	<i>S. occidentalis</i> (2000 mg/kg bw)
Urea (mmol/l)	0.37 ± 0.026	0.37 ± 0.015	0.27 ± 0.023*
Creatinine (mmol/l)	3.66 ± 0.33	3.33 ± 0.33	3.66 ± 0.33
Blood glucose (mmol/l)	0.97 ± 0.033	1.03 ± 0.039*	0.96 ± 0.045
Cholesterol (g/l)	0.58 ± 0.017	0.62 ± 0.014	0.56 ± 0.015
ASAT (U/I)	161.5 ± 1964	134.7 ± 1.76*	131.3 ± 1.85*
ALAT (U/I)	40.33 ± 0.88	38.33 ± 1.20	36.33 ± 0.88

ALAT, alanine aminotransferase; ASAT, Aspartate aminotransferase. In the Table, on each line *: significant difference at $P < 0.05$; Absence of asterisk (*) on values indicates no significant difference $p > 0.05$, ANOVA, Newman–Keuls test.

Table 6.
Effects of total methanolic extract of *Tithonia diversifolia* and *S. occidentalis* on biochemical parameters of female rats.

Parameters	Control (distilled water)	<i>T. diversifolia</i> (2000 mg/kg bw)	<i>S. occidentalis</i> (2000 mg/kg bw)
WBC ($10^3/\mu\text{l}$)	19.36 ± 1.16	20.08 ± 0.86	21.30 ± 1.35
RBC ($10^6/\mu\text{l}$)	8100 ± 0.38	7120 ± 0.27	7515 ± 0.255
HGB (g/dl)	12.93 ± 0.26	12.20 ± 0.52	12.90 ± 0.7
HCT (%)	38.90 ± 0.83	37.70 ± 0.77	38.10 ± 0.9
MCV (fL)	51.50 ± 0.81	51.53 ± 0.58	51.60 ± 0.7
MCH (pg)	17.20 ± 0.23	17.13 ± 0.12	17.05 ± 0.35
MCHC (g/dl)	31.53 ± 0.37	32.71 ± 0.32	33.00 ± 0.2
PLT ($10^3/\mu\text{l}$)	723.7 ± 17.07	710.3 ± 16.29	705.5 ± 14.5
NEUT (%)	21.45 ± 0.65	21.45 ± 0.88	21.12 ± 1.34

WBC, white blood cells; RBC, red blood cells; HGB, hemoglobin; HCT, hematocrit; MCV, mean blood volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, blood platelets; NEUT, neutrophil. The absence of an asterisk (*) on the values indicates that there is no significant difference $p > 0.05$, ANOVA, Newman–Keuls test.

Table 7.
Effects of methanolic extract of *Tithonia diversifolia* and *S. occidentalis* on hematologic parameters of female rats.

3.8 Effects of methanolic extracts on histological parameters

The histopathological study performed on the liver, kidney, and heart revealed no structural abnormalities (inflammation, hepatic cell necrosis, and apoptosis) in the rats treated with different doses of total methanolic extracts of *T. diversifolia* and *S. occidentalis*, compared to the sections of the organs taken from the control (**Figure 4**).

4. Discussion

In the laboratory, methanolic extracts of the leaves of *S. occidentalis* and *Tithonia diversifolia* showed contact insecticidal activity against the termite *Ancistrotermes*

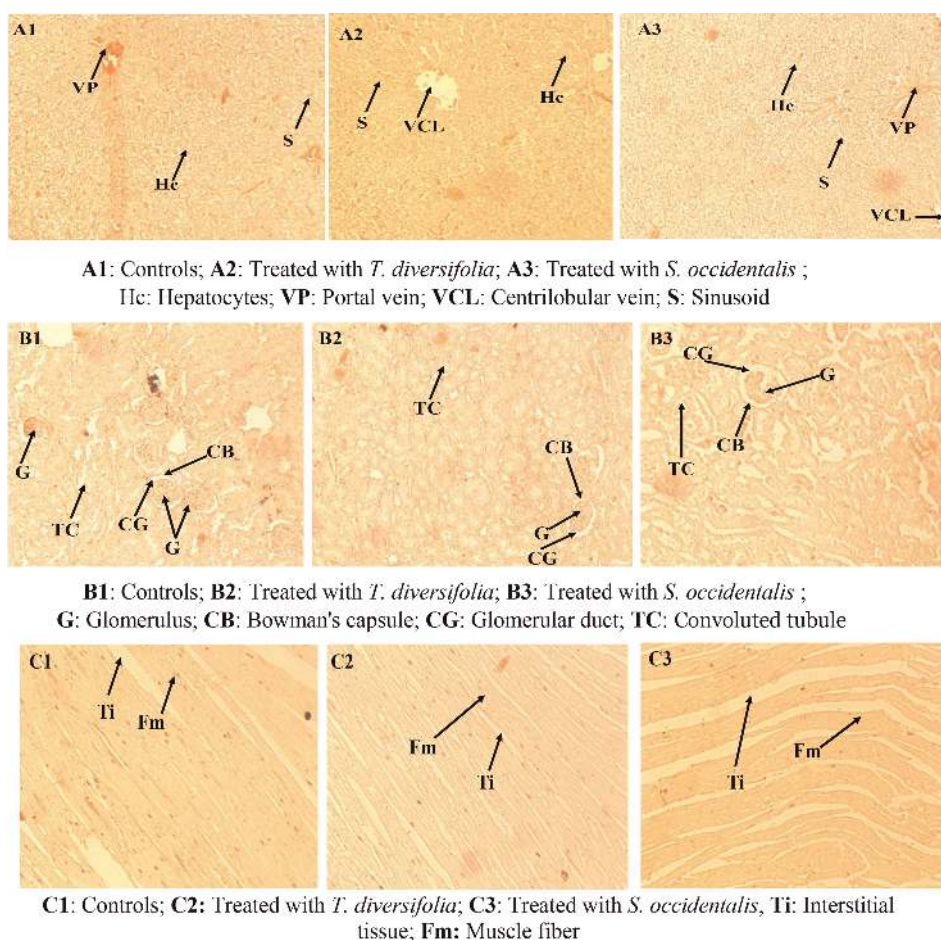


Figure 4.
Histological sections of rat liver (A), kidney (B), and heart (C). Staining: Hematoxylin and eosin, $G \times 100$.

cavithorax. Several studies have shown contact insecticidal activities on termites by plant extracts. Extracts of *Azadirachta indica* and *Carica papaya* showed contact insecticidal activity on *Macrotermes bellicosus* [14, 18]. *C. papaya* extract also showed contact insecticidal activity on *Coptotermes formosanus* Shiraki [11]. *T. diversifolia* extract also showed insecticidal activity on *Ancistrotermes* sp. [19].

The mortality rate of *Ancistrotermes cavithorax* termites observed after treatment with total methanolic extracts increases proportionally with concentration and time. Thus, the higher the dose, the more toxic over time and the more active the products are. These results therefore suggested that methanolic extracts of *S. occidentalis* and *T. diversifolia* have their optimal action on *Ancistrotermes* termites at high dose and long term. The work of Diby et al. [19] also showed that the aqueous extract of *T. diversifolia* is toxic at high dose and long term on *Ancistrotermes* sp. These results showed that the toxicity of methanolic extract of *T. diversifolia* is significantly higher than that of *S. occidentalis* on *Ancistrotermes cavithorax* termites.

The LC₅₀ values confirmed that the methanolic extract of *T. diversifolia* (LC₅₀ = 29.76 mg/l) would be more active on *Ancistrotermes cavithorax* termites than the methanolic extract of *S. occidentalis* (LC₅₀ = 84.9 mg/l).

The mortality of termites recorded during the experiment shows that the biological efficacy of the methanolic extract of *S. occidentalis* and *T. diversifolia* seems evident but is not immediate after treatment. This slow action could be due to insufficient active concentration and insufficient penetration of the product through the cuticle or oral apparatus of the insects [20, 21]. This observation is confirmed by the 50% mortality rates of *Ancistrotermes* termites obtained before 48 h with the total methanolic extract of *S. occidentalis* and *T. diversifolia*.

The extracts also seemed to act by inhalation. Indeed, inhalation with the methanolic extract of *S. occidentalis* and *T. diversifolia* significantly reduced the population of *A. cavithorax* termites tested. Contact and inhalation would thus be the two essential ways to the effectiveness of the two extracts on the *Ancistrotermes* termite.

For the barrier soil tests, the sensitivity of the *A. cavithorax* termite workers did not allow the test to continue over a long period. Only 10 days of continuous testing provided results. The results confirmed the toxicity of the methanolic extract of *T. diversifolia* and *S. occidentalis*, as well as the anti-appetitive action of these extracts on consumption. In this study, the extracts used as a barrier soil did not prevent termites from coming into contact with the food after the treated substrate chamber. Workers mortality rates in the treated substrate chambers and in the food chambers located after the treated chambers confirm that the termites came into contact with the food. Tahiri [11] and Diby et al. [19] showed that *C. papaya* and *T. diversifolia* macerates used as barrier soil did not prevent *C. formosanus* and *Ancistrotermes* sp. termites from contacting the food. In our trials, we did not obtain total mortality. Blaske and Hertel [22], Tahiri [11], and Diby et al. [19] also showed that compounds derived from *Jatropha curcas*, *A. indica*, *C. papaya*, and *T. diversifolia* tested as barrier soil did not cause total mortality in *Coptotermes* sp. and *Ancistrotermes* sp.

Although termites have penetrated the barrier soil (treated soil), food in the food chamber is not consumed. In nearby untreated food chambers, termite consumption is affected and is much lower than in controls. Zhu et al. [23] and Tahiri [11] showed that other botanical insecticides tested on *Coptotermes* sp. also affect termite foraging.

The acute in vivo toxicity study of the two plant extracts, 14 days after oral administration of methanolic extracts of *T. diversifolia* and *S. occidentalis* to different batches of female rats, did not record any deaths at the single dose of 2000 mg/kg body weight. The toxicity of the chemicals was determined by the LD50 value. The administered dose is 2000 mg/kg body weight, the LD50s would therefore be higher than 5000 mg/kg by the oral route. According to the OECD Globally Harmonized System of Classification and Labeling of Chemicals (2001), methanolic extracts of *T. diversifolia* and *S. occidentalis* can be classified as nonclassified category 5. The dose of 2000 mg/kg body weight would be below the maximum tolerated dose (MTD), and these extracts would therefore be nontoxic.

The results obtained in this study corroborate those obtained by other authors on the same plants. Thus, it was shown that the total methanolic extract of *S. occidentalis* leaves was not toxic in rats at a dose of 1000 mg/kg body weight 72 h after oral administration [24]. In contrast, the 70% ethanolic extract of *T. diversifolia* leaves showed a dose- and time-dependent toxic effect. This effect was reversible on the kidneys and liver, with no discernible adverse effect on the morphology of the heart, spleen, and brain [17].

Relative organ weights showed no significant difference from the control at the end of 14 days of treatment. This result suggested that the single dose of 2000 mg/kg body weight of methanolic extracts of *T. diversifolia* and *S. occidentalis* would not influence organ morphology.

Analysis of biochemical parameters showed that the creatine level of the rats did not change significantly ($P > 0.5$) compared to the control at the end of 14 days of treatment at the dose of 2000 mg/kg body weight. Only urea level decreased significantly ($P < 0.5$) in rats treated with methanolic extracts of *S. occidentalis* compared to the control. Urea and creatinine are markers of renal function that indicate glomerular filtration rate, concentrating, and diluting capacity [25]. Increased values of these markers indicate kidney dysfunction [26]. The reduction in serum urea levels in the present study has no risk to the kidney. The single dose of 2000 mg/kg bw of methanolic extracts of *T. diversifolia* and *S. occidentalis* leaves do not show any hazard on kidney.

ALAT activity also showed no significant change ($P > 0.05$) with the administration of the methanolic extracts of *T. diversifolia* and *S. occidentalis* compared with control rats. In contrast, there was a significant ($P < 0.05$) reduction in ASAT activity compared with control rats. ALAT is more specific for liver damage, whereas ASAT is predominantly present in the liver, muscle, heart, kidney, brain, and pancreas [27]. Serum levels of these enzymes increase in myopathy, rhabdomyolysis, myocardial infarction, hepatitis, or hepatic cirrhosis. The significant decrease in ASAT activity indicates that the administration of methanolic extracts of *T. diversifolia* and *S. occidentalis* at the dose studied would not cause either liver toxicity or cardiac damage. This fact is comparable to the previous diagnosis of Ejelenou et al. [25], according to which the administration of saponin from the leaves of *T. diversifolia* at the dose 20–100 mg/kg body weight does not cause any risk to the hepatic, renal, and cardiac tissue. In the case of this study, it could be that the extracts have a hepatoprotective action at the dose of 2000 mg/kg of body weight, which would lower the ASAT level in the liver. Indeed, several studies have highlighted the hepatoprotective role of plant flavonoids [28, 29].

Macroscopic and microscopic examinations of the liver, kidney, and heart of animals treated with different doses of methanolic extracts of *T. diversifolia* and *S. occidentalis* showed a normal structural architecture compared to the control. This confirmed that single oral administration of 2000 mg/kg body weight of *T. diversifolia* and *S. occidentalis* extracts, after 14 days, does not result in any harmful changes and morphological disorders. The absence of structural abnormalities, inflammation, hepatic cell necrosis, and apoptosis of the liver, kidney, and heart in all rats treated with methanolic extracts of *T. diversifolia* and *S. occidentalis* confirmed the results of the relative organ weights and biochemical parameters. Thus, methanolic extracts of *T. diversifolia* and *S. occidentalis* would not be toxic at a single dose of 2000 mg/kg bw in adult rats after 14 days.

5. Conclusion

This toxicity study showed that methanolic extracts of *T. diversifolia* and *S. occidentalis* were toxic by contact and inhalation on the crop pest termites, *Ancistrotermes cavithorax*. Used in barrier soil, these extracts were anti-palatable but not repellent. The research on the toxicity of these extracts on mammals, here the rat, shows that according to the OECD guideline, these two extracts were classified as nontoxic. The confirmation by the hematologic and biochemical study did not show any proven toxicity of the extracts at the single dose of 2000 mg/kg of body weight. The histopathological study carried out on the liver, the kidney, and the heart did not reveal any structural abnormality (inflammation, hepatic cellular necrosis, apoptosis)

and confirmed the nontoxicity of these two extracts. These two plants could therefore be recommended to farmers as biopesticides in order to treat attacks by *Ancistrotesmes* in their crops without danger to their health.

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