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Repellent and toxic of macerât of *Tithonia diversifolia* (Hemsl.) gray (Asteraceae) on *Ancistrotermes* sp. at the laboratory

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Abstract
Biological tests conducted at the laboratory of Entomology and Agricultural Zoology of the National Polytechnic Institute Houphouët-Boigny (INP-HB) of Yamoussoukro (Côte d'Ivore) allowed to evaluate the termitive and toxic effect of *Tithonia diversifolia* macerate on Ancistrotermes sp. *Tithonia diversifolia* macerate prepared at 10 and 13% (100g / L and 130g / L) was used to evaluate contact, consumption and barrier soil toxicity. The direct toxicity test shows that the mortality rate in the tests is higher than that of the controls and this as a function of the macerate dose. The 13% concentration is more toxic than the 10% concentration (LC50 is 16,020 mg / 13% versus LC50 42,905 mg / L at 10%). At the lowest dose (10 µl), 50% of the mortality was reached from the 8th day with the 13% solution against 11% with the 10% solution. The consumption test reveals that termites consume very little treated papers (1.25 mm2 / worker for the dose of 10% and 0.50 mm2 / worker for that of 13% against 12.13 mm2 / worker in the witnesses). In the soil barrier (excavation) test, the total mortality rate obtained in the trial arenas of 10% and 13%, respectively, is 2.6 and 2.8 times higher than that of the control arenas, respectively. The excavation test confirms that the product is toxic. The tunnel constructions were observed in the introduction chambers and in the substrate chambers both in the tests and in the controls. The pieces of filter paper in the food chambers located after the treated substrate chambers were not consumed in the test arenas dosed with 13% extract of *T. diversifolia*. The macerate of *Tithonia diversifolia* does not prevent the termite from coming into contact with the treated soil but reduced or completely prevent consumption in the food chambers contiguous to the treated substrate chambers. *T. diversifolia* seems to be non-repellent but anti-appetizing for termites.

Keywords: termite, toxicity, test excavation, *Tithonia diversifolia*

1. Introduction
Pest termites are responsible for damages to woody plants and crops. According to [1], approximately 3106 species have been described to date, but only 10% of these organisms are known to be harmful to living plants and buildings [2]. Among the termites pests, *Ancistrotermes sp* is responsible for damages to crops such as Hevea [3] and Nerica1 rice [4]. In the face of these attacks, chemical insecticide products are the most used to reduce or kill these insect pests. On the other hand, the use of chemicals on crops is not without side effects on living organisms and the disturbance of ecological niches (mammalian toxicity, pest resistance to insecticides and ecological risks) [5]. The adverse effects of products on the environment require new means of effective control and the establishment of new methods of control. Among the new control methods, the use of insecticidal plants seems promising for a fight respectful of the environmental conditions. The objective of this study is to evaluate the toxicity of leaf macerate of *Tithonia diversifolia* on termite *Ancistrotermes sp*. It is specifically to determine the toxicity by contact, by consumption and to verify the mode of action of this macerate in a barrier soil by the excavation test.

2. Material and methods
2. 1 Plant material
Tithonia diversifolia (Hemsl.) Gray (1883) is a stoloniferous perennial herb that can reach 2 to 3 m in height (Fig 1). The species was harvested early in the morning along the roads of the Yamoussoukro Agronomic School (Côte d'Ivoire).

2. 2 Animal material
The termite *Ancistrotermes sp* was harvested live on the experimental plot of the School of Agronomy of INP HB (Côte d'Ivoire) and used for laboratory tests.

2.3 Preparation of Macerat
The leaves of *Tithonia diversifolia* were collected early in the morning. The drying of the leaves lasted three (3) weeks, in the shade in a room designed for this purpose and on black plastic sheeting. A mixer was used to powder the dried leaves.
For the preparation of the macerate or aqueous filtrate of the leaves of *Tithonia diversifolia*, for our tests, the concentrations of 10 and 13%, ie 100 and 130 g / L, were used. One thousand forty (1040) grams of powder were macerated for 24 hours in 8 liters of water. After maceration, double filtration was

carried out with a 1 mm mesh water sieve and then with a white "percale" fabric (Fig 2). The macerate obtained is determined at 13%. Dilution was made to obtain the 10% solution.

2.4 Direct toxicity test of *Tithonia diversifolia* on *Ancistrotermes*

The direct toxicity tests, with 100 workers of Ancistrotermes sp, are carried out in a petri dish containing 7 g of soil moistened with 2 ml of distilled water (Fig 3) [6, 7, 8]. Using a micropipette, the doses from the solution obtained after maceration, prepared at 10 and 13% of *T. diversifolia* macerate, are poured and mixed with the soil. The *T. diversifolia* extract solution is tested at four doses (10, 20, 50 and 100 µL). Each dose is repeated 3 times. The dead workers are counted every 2 hours and after 24 hours the count is done every day.

2.5 Consumption toxicity test

For consumption tests, the doses are poured on 4 cm2 pieces of Watman N°4 filter paper (Fig 4) [6, 7, 8]. After the treatments, the dishes are dried in the open air for 1 hour.

One hundred (100) *Ancistrotermes sp* workers are then introduced into this closed device. The *T. diversifolia* extract solution is tested at four doses (10, 20, 50 and 100 µL) [8]. Each dose is repeated 3 times for all tests. Each control box is treated with distilled water. The dead workers are counted every 2 hours and after 24 hours the enumeration is in the day. The percentage of mortality is determined.

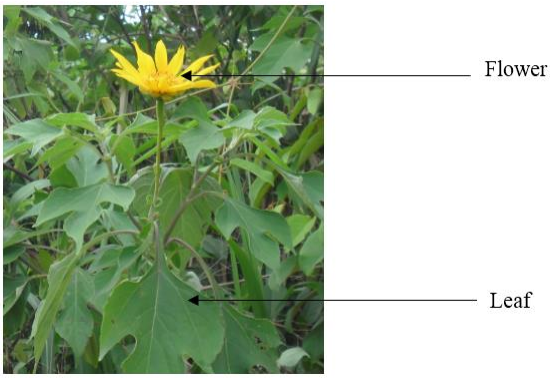

Fig 1: Leaf and flower of *Tithonia diversifolia*

Fig 2: *Tithonia diversifolia* macerate dosed at 10 and 13%.

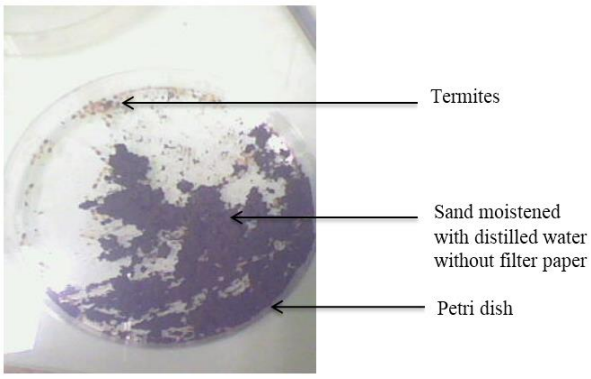

Fig 3: Direct toxicity test (Witness)

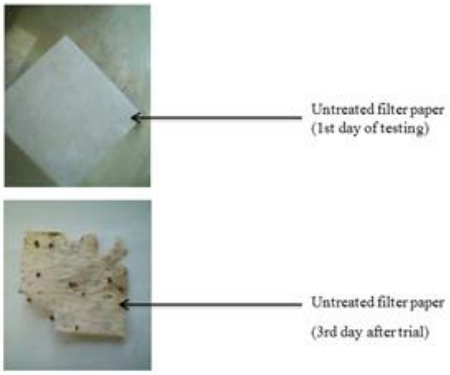

Fig 4: Consumption toxicity test (control test)

2.6 Excavation test

The choice of soil or barrier soil test with soil moistened with 10 and 13% *T. diversifolia* extract is a more realistic simulation of the product's efficacy in the wild (Fig 5) [9, 8].

Nine boxes (chambers) made of plastic (5 cm diameter x 3.5 cm high), closed by plastic covers, are used by arena. Each arena consists of an introductory chamber containing the sieved soil of the nest, four substrate chambers containing the soil from the nest and four food chambers each containing 2 pieces of 4 cm^2 Watman No.4 filter paper which were used for termite feeding. The rooms are connected to each other and to the introduction room located in the center of the arena, by transparent tubes (7 cm in length, LABELL Ch / Fr No. 20, Overall length 49 Inch). In each test, only one substrate chamber is treated by arena as barrier soil (50 g of soil moistened with 7.5 ml of the product). The other untreated substrate chambers contain 50 g of soil moistened with 7.5 ml of distilled water. In the control arenas, the 4 substrate chambers are untreated.

Three hundred (300) large *Ancistrotermes sp* workers are weighed before being introduced into the arena by the introductory chamber [8]. They will remain isolated for 24 hours in this room (clamped connection tubes) to acclimatize, before allowing them to circulate freely in all rooms of the arena. The arena is kept in a room at 27 ° C.

Three repetitions with 300 termites are performed for each test with *T. diversifolia* extract and for each corresponding control [8].

After 10 days of continuous exposure to the product, surviving workers are counted in all rooms of the arena (treated as

untreated) and their total weight calculated. The task of building galleries and the food consumption of workers are compared between treated and untreated.

2.7 Statistical treatment

The variance analyzes were carried out using SPSS software version 17.0 and the determination of the LC50 was made by probit analysis with the software "XL STAT version 2013" according to the mortalities obtained after 24 hours of testing for each dose.

For comparison of the averages of the different results with the control, the Duncan test was applied. The probability threshold chosen is P <0.05.

3. Results

3.1 Effect of *T. diversifolia* macerate on termites of the genus *Ancistrotermes*

3.1.1 Direct toxicity

Comparison of the 4 doses tested gave an LC50 of the 10% solution of 42.905 mg / L (ie 429.05 µL) significantly greater than that of the 13% solution of 16.020 mg / L (ie 122.06 µL).) (Anova, P <0.001) (Table I). The toxicity of the macerate dosed at 13% of the solution is greater than that of 10%. In addition, the mortalities of the 4 doses (10, 20, 50 and 100 µL are respectively 1; 2; 5 and 10 mg of extract / L), for both the solution dosed at 10% and for the solution at 13% (1.3, 2.6, 6.5 and 13 mg of extract / L) were not significantly different (P> 0.05). At the lowest dose (10 µL), 50% of the mortality was reached by the 8 th day with the 13% solution against 11 µ with the 10% solution (Table II).

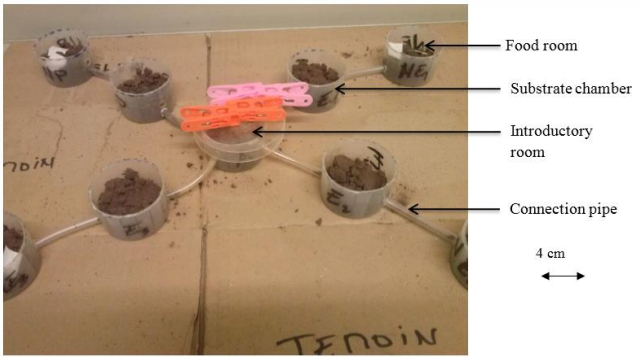

Fig 5: Excavation test (Witness)

3.1.2 Toxicity by consumption

Untreated pieces of filter paper and those treated with the 10 and 13% extract are visited by the termites, as shown by the presence of earth veneers (Table III). On the other hand, termites consume very little of the treated papers (1.25 mm2 / worker for the dose of 10% and 0.50 mm2 / worker for that of 13%). In contrast, the average paper surface consumed in the control is 12.13 mm 2 / worker (Table III). The correlation test between mortality and consumption is not significant (r = 0.061, P = 0.852, N = 12 for the 10% test and r = 0.185, p = 0, 566, N = 12 for the test). test at 13%). Consumption is not the only factor responsible for the death of termites. The toxic effect of T. diversifolia macerate is therefore not only related to the consumption of leaf pieces by termites.

3.1.3 Excavation test

In the trial and control arenas, after 10 days of continuous exposure of 300 workers to a soil moistened with 10 and 13% of *T. diversifolia* extract, the mortality rate of termites in the trials is significantly higher than that obtained in controls (P <0.05) (Table IV). Increasing the concentration of *T. diversifolia* extract did not have a significant influence on termite mortality in the trials (Table IV). Mortality is unevenly distributed in the 4 substrate and food chambers at both trial and control levels. The mortality rate in the substrate and food chambers of the trials is greater than the mortality rate of the controls.

In the introduction chambers, the mortality rate of the workers in the trials is significantly higher than the control (Table V).

Table 1: Lethal Concentration 50 (LC 50) of *Tithonia diversifolia* leaf macerate at 10 and 13% on *Ancistrotermes* workers

Treatments (leaf macerate)	DL 50 à 24h (µL)	CL 50 à 24h (mg/L)
10%	429,05 ±35,753 b	42,905
13%	122,06 ± 35,753 a	16,020
F test Probability	18,57P <0,001	

The LC50 calculated by the probit analysis is based on the mortality obtained after 24 hours. In each treatment 100 termites are tested with 4 repeated concentrations 6 times (10, 20, 50 and 100 µL) (N = 2400).

Table 2: Lethal time 50 (TL 50) of *Tithonia diversifolia* macerate at 10 and 13% on *Ancistrotermes* workers

doses (µL)	TL50 à 10% (Jours)	TL50 à 13% (Jours)
10	11	8
20	11	7
50	6	6
100	4	4

Time required to obtain 50% of death for each solution

Table 3: Effect of *T. diversifolia* macerate on the harvesting activity of *Ancistrotermes* workers

Concentration of *T. diversifolia* macerate	Cumulative plating surface (mm2 / ov)	Cumulative consumed paper area (mm2 / ov)
control	14,75 ± 7,95 a	12,13 ± 1,51 b
10%	6,75 ± 6,08 a	1,25 ± 0,47 a
13%	5,88 ± 4,87 a	0,50 ± 0,28 a
F test	0,578	48,47
Probability	P>0,05	P<0,001

Mean of 4 repetitions ± standard deviation (N = 400). Within the same column, the values followed by the same letters are not significantly different at the 5% threshold

Table 4: Mean mortality of Ancistrotermes workers after 10 days of exposure to soil moistened with *Tithonia diversifolia* macerate at 10% and 13% (choice of excavation test).

% of humidification of the ground	Mean mortality and standard deviation
10%	27,85 ± 5,59 b
13%	29,26 ± 6,62 b
Distilled water	10,41 ± 1,49 a
F test	4,27
Probability	P<0,05

The values followed by the same letters are not significantly different at the 5% threshold (Anova, Spss). Average of 3 repetitions + standard deviation (N = 900). (P <0.017)

Table 5: Mean Mortality Rate of *Ancistrotermes* sp Workers in Main or Introductory Chambers After 10 Days of Exposure to Wet Soil with 10 and 13% *Tithonia diversifolia* Water Extract (Excavation Test) choice).

% of soil moisture	Mean mortality and standard deviation
10%	94 ± 5,568 **b**
13%	122 ± 10,14 **c**
Distilled water	26 ± 7,21 **a**

The values followed by the same letters are not significantly different at the 5% threshold (Anova, Spss). Average of 3 repetitions + standard deviation (N = 900). (P <0.000)

The tunnel constructions were observed in the introduction chambers and in the substrate chambers both in the tests and in the controls.

The food chambers in the trials have very few tunnels. The presence of some grindstone formations in some substrate chambers was observed in control arenas.

In the 10 and 13% trial arenas, after 10 days of exposure, the mortality rate in the introduction chambers is higher than that of the control arenas (P <0.05) (Table V). The total mortality rate obtained in the trial arenas of 10 and 13%, respectively, is 2.6 and 2.8 times higher than that of control arenas.

The mortality rate in the introduction chambers of the trial arenas, is higher than that of the substrate and food chambers. Moreover, this rate is higher in the arena at 13% than at 10%. The lowest mortality rates are observed in substrate chambers containing *Tithonia diversifolia* macerate.

In the trial arena (containing a treated chamber), the consumption rate of the workers in the food chambers is lower than that of the controls (P <0.05) (Table VI). Despite the different possibilities available to termites, one (1) food room out of four in the trial arena assayed with 10% extract of *T. diversifolia* is used. The pieces of filter paper in the food chambers located after the treated substrate chambers were not consumed in the test arenas dosed with 13% extract of *T. diversifolia*.

In the control arena, two (2) out of four food rooms are used. The presence of grinding wheels is observed in only two food chambers.

Table 6: Aggregate consumption of *Ancistrotermes* workers after 10 days exposure to soil moistened with 10% and 13% *Tithonia diversifolia* macerate (choice of excavation test).

% of humidification of the ground	Cumulative consumption (mm^2) and standard deviation
10%	1 ± 1,75 a
13%	0 ± 0 a
Distilled water	142 ± 48,77 b
F test	25,27
Probability	P<0,01

The values followed by the same letters are not significantly different at the 5% threshold (Anova, Spss). Average of 3 repetitions + standard deviation (N = 900). (P <0.001)

4. Discussion

The toxicity test confirmed to the laboratory the insecticidal effect of leaf macerate of *T. diversifolia* on the termite *Ancistrotermes sp*. This species has been shown to be sensitive to the extract of *T. diversifolia* and confirms the work of Diby *et al*. (2015) [10] carried out on this species in the field with the extract of *Tithonia diversifolia*. *T. diversifolia* acts on contact and the LC50 has shown that the higher the concentration, the higher the mortality.

The consumption toxicity test revealed that the extract of *T. diversifolia* seems to be an inhibitor of food intake with regard to termite at the experimental concentrations used. According to Adoyo *et al.* (1997) [11], leaves of *T. diversifolia* contain phenolic compounds (tannin) and other substances. Certainly, tannin would be responsible for the anti-appetizing effect of *T. diversifolia* and would prevent termite from taking food. Some authors [12, 13] have shown the insecticidal, larvicidal and repellent effect of tannin contained in insecticidal plants. The use of bait macerate against this insect can therefore pose problems at high concentration.

For the excavation test, the sensitivity of the termite *Ancistrotermes* did not allow to continue the test over a long period. Only 10 days of continuous testing yielded results. The results of the excavation test confirm the toxicity of *T. diversifolia* and its anti-appetizing effect on consumption. This study showed that the macerate (10% and 13% concentrate), used as barrier soil, does not prevent the termite from coming into contact and crossing it. The presence of tunnel in treated soils confirms it. The workers who were able to cross the treated substrate chambers died in the food rooms while very few died in the treated rooms. These results are similar to those obtained by Tahiri (2012b) [8] on termite *Coptotermes formosanu* with *Carica papaya* macerate. Total mortality could not be observed in the trial arenas. These results are similar to those obtained by Bläske and Hertel (2001) [14]; ACDA (2009) [15]; Tahiri (2012b) [8], who showed that compounds derived from *Jatropha cursas*, *Azadirachta indica*, *Carica papaya* and isoborneol tested as barrier soil did not cause total mortality in *Coptotermes*.

Table 7: Mortality of *Ancistrotermes* workers after 10 days of exposure to soil moistened with 10% and 13% *Tithonia diversifolia* macerate (choice of excavation test).

% of humidification of the ground	Mean mortality and standard deviation
10%	27,85 ± 5,59 b
13%	29,26 ± 6,62 b
Distilled water	10,41 ± 1,49 a
F test	4,27
Probability	P<0,05

The values followed by the same letters are not significantly different at the 5% threshold (Anova, Spss). Average of 3 repetitions + standard deviation (N = 900). (P <0.017)

Although the termite is able to penetrate the barrier soil (treated soil), food in the food chamber is not consumed. The consumption of termites in neighboring untreated food rooms is lower than that of controls. Some authors ([16, 8]) have shown that botanical insecticides tested on *Coptotermes sp* affect the foraging of termites.

In this study, the mortality rate in the introduction chambers is high in the test arenas compared to control arenas. This behavior may be due to the toxic effect of the tannin contained in the *T. diversifolia* extract. The termite having been in contact with the product has certainly preferred to take refuge

in the introduction chamber.

In the control arena, the product not being present, the high mobility in the other chambers was observed therefore resulting in low mortality in the introduction chamber. This result shows that the product affects the movement of termites in the arena and the construction of galleries.

5. Conclusion

The results obtained confirm the toxicity of *Tithonia diversifolia* macerate. The termite can come into contact with the product, which is an advantageous point in the fight against underground pest termites such as *Ancistrotermes*, mobile and living inside some nests of other termites. These results also show that the macerate is not repellent.

Preventatively, soil treatment around a crop would significantly reduce termite attack and consumption and reduce the mobility of the termite population as well as the construction of galleries on the plants. On the other hand, on this species of termite, although toxic, the extract does not eradicate the population of termite. The excavation test, which is a simulation of the actual conditions of the termite before field application, confirmed the results of the toxicity and consumption tests. This natural insecticide could be very promising for farmers. This study paves the way for the search for biopesticides or biocides in the fight against plant pests.

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