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Pesticidal properties and screening of extracts of *Pueraria phaseoloides* (Roxb.) Benth. (Fabaceae) on *Macrotermes bellicosus* (Rambur) (Isoptera; Macrotermitinae)

Tahiri Annick
Laboratory of Endocrinology and Reproductive Biology, Faculty of Biosciences, University of Félix Houphouet Boigny, 22 BP 582, Côte d'Ivoire

Abstract
Toxicity, the lethal dose, the mode of action, efficacy and persistence of the chemical composition of alcoholic, hexanic and aqueous extracts of the leaves of the Legume *Pueraria phaseoloides* were considered on the pest species of termite *Macrotermes bellicosus*. The contact toxicity of extracts of the termite is low and does not cause total mortality of the populations tested. The persistence of efficiency of the extracts is about 1.4 to 3.4 days. The hexane extract of leaves, the most toxic, has an LD50 of 158.6 $\pm$ 0.41 mg / l. Its effectiveness does not appear to be related to its inhalation and ingestion by the insect. However, in contact, this extract has an inhibitory effect of food intake. It contains two main secondary compounds, flavonoids and terpenoids.

Keywords: termite, *Pueraria phaseoloides,* pesticidal properties, screening

1. Introduction

Research and development are mainly focused on the active insecticides for the fight against insect pests. Termites have acquired the status of major pest in tropical agro ecosystems. Soil treatment by termiticides is the standard technique to fight against termites pests, without real success and perverse side effects (toxicity to mammals, pest resistance to insecticides and environmental risks) [1]. With restrictions on the use of conventional insecticides, the growing need to find alternative methods reliable and environmentally better for management systems and agricultural pests is imposed. Among the alternative strategies, use in singular form as insecticide plants seems promising.

We wanted to develop the local knowledge of plant pesticidal properties. We have focused our work towards research of biopesticides. In Côte d'Ivoire and Cameroon, the cover crop Legume *Pueraria phaseoloides* is used in perennial crops, to fertilize and protect the soil against erosion, but also in protection against termite attack [1, 2, 3]. In some countries, this legume was used as forage used for feeding cattle [4]. Also, we checked the basis of traditional use of this plant in the country in anti-termite and specifically seek its mode of action of termites. The choice of this stu dy is based on the following observation: The traditional herbal for the fight against insect pests are supplanted by chemical inputs. The overall objective of this study is to propose an alternative of fighting the use of chemical inputs, more efficient, more accessible to farmers, without threat to the environment and to humans and less expensive.

More specifically, it is:

- To study whether direct toxicity of different aqueous, alcoholic and hexanic of leaves extracts of *Pueraria phaseoloides* on adult workers of a pest species of termite *Macrotermes bellicosus* Rambur;
- To determine:

If termite mortality may or may not result in a consumption of the extract;

If the extract, not in contact with the termite, is toxic (inhalation test);

If the workers, when choice is given, are able to detect the product and avoid (choice test);

- To assess the lethal dose LD50 in 24 hours;
- Determine the continuing effectiveness of active extracts of this plant;
- Identify the main classes of secondary metabolites contained in the most active extract from this plant.

2. Materials and methods

2.1 Study site

The work was conducted at the University of Félix Houphouet Boigny and Campus, located in the east of the city of Abidjan, Côte d'Ivoire.

2.2 Plant material

Our choice fell on the Legume *Pueraria phaseoloides* (Roxb.) Benth. (Fabaceae) var *javanica* (Benth.) Baker, 1864 (Fabaceae), commonly found in Côte d'Ivoire. This cover crop is often described as toxic and repellent against termites [1, 2, 3]. However, few works develop its pesticidal properties.

2.3 Animal material

Macrotermes bellicosus (Rambur 1842) of the *Macrotermitinae* subfamily was selected for biological testing because of its impact on many crops, its abundance in the study area and its aboveground nest that allows capturing in large numbers of workers. In this work, the workers studied are from the same colonies from the same nest which we have found on the soil of the university campus. The workers collected from this nest was brought to the laboratory and species identification was confirmed by an identification key [5].

2.4 Preparation of total extracts
The plant is harvested from May to September 2005 and 2006. The aqueous extracts, methanol and hexane leaves, are made under the method of classical successive extraction by conventional solvents of different polarities [6].

2.5 Calculating the rate (in %) of the extraction yield
The yield to successive extraction (*Y*) is defined as the ratio of the amount of the extract (*E*) on the dry matter of the product (*MP*):

$$Y = E / MP \times 100$$

2.6 Preparation of formulations
A stock solution of 10% was prepared from each dried extract with the corresponding solvent before being tested on 1 g fresh weight of small workers (SW) of the termite *M. bellicosus* adult (differentiated by the size of the head, smaller than that of major workers) or small workers of 136, at doses of 10 - 20 - 50 and 100 µl per box, respectively 1 - 2 - 5 and 10 mg extract / l. The control is treated the same doses of the corresponding solvent.

2.7 The biological tests
Four biological tests are realized according to the protocols of Delgarde and Rouland-Lefèvre (2002). (I) The direct toxicity test measures the response of termites in soil treated with the insecticide. (II) The toxicity by consumption to determine if the mortality of small workers was or was not resulting from consumption of the insecticide and the precise extent of consumption of the product in this mortality. (III) The inhalation toxicity study if the fumes act as toxic insecticide. (IV) Avoidance of the extract to determine whether the workers, when choice is given, are able to detect and avoid the product.

The tests was performed at ambient laboratory temperature between 27 and 28 °C. Toxicity tests and by direct consumption was done in a small rectangular plexiglass box of 95 x 65 x 20 mm high containing 7 g of soil moistened with 2 ml of distilled water. The inhalation tests and avoidance was performed in a large plexiglass box of 180 x 120 x 70 mm high containing 17 g of sieved soil and moistened with 5 ml of distilled water. Using a micropipette, the plant extract was poured on the soil (for direct toxicity tests) or on pieces of Whatman No. 1 of 4 cm^2 (for consumption tests, inhalation and avoidance). After treatments, the dishes were dried in open air for 1 hour and solvent evaporated. The small workers of *M. bellicosus* was then introduced into these devices that are closed and do not allow air circulation. Each extract solution was tested with four doses cited. Each dose was repeated ten times for all tests. Each control box was treated with the corresponding solvent. Small dead workers were counted in every 24 hours. The mortality rate was calculated. The LD50 is calculated by Probit analysis based on the mortalities obtained after 24 hours on various doses (Statistica, 2001) [8]. The surface of each Whatman paper covered with soil and that consumed (mm^2/worker) per day was measured with an ocular micrometer adapted to a magnifying glass. The amount of extract ingested per worker per day (in ppm) was calculated.

2.8 Persistence of efficiency (in days) of the most active extract
In the small plexiglass box, the soil is covered in the most effective dose of the extract. Small workers are removed and replaced every 24 hours for 7 days by the new ones. Control boxes were treated with the corresponding solvent. Small dead workers are counted until the amount in the tests and the control boxes are not significantly different. The mortality rate is calculated.

2.9 Calculation of percentage of mortality
The mortality percentage (*MP*) is calculated using the ratio of the number of dead individuals observed on the total number of termites.
MP = observed mortalities / total termites x 100

2.10 Chemical tests for the characterization of secondary metabolites extracted from the plant
A phytochemical screening was carried out on leaves of *P. phaseoloides* to highlight the major chemical groups. Chemical tests for the characterization of alkaloids, flavonoids, saponins, tannins and terpenoids were performed using the technique of Harbone [7].

2.11 Statistical analyzes
Data collected during the biological tests are processed using the Statistica software [8]. The box plot, bootstrap estimation, Probit analysis, the nonparametric Kruskal-Wallis (at 5%) and correlation tests are implemented here.

3. Results
3.1 Extraction yield of successive leaves of *P. phaseoloides*
The successive quantities extracted by each of the three solvents, expressed as a percentage of the total amount extracted, are higher in alcohol and aqueous solvents (respectively 6 and 5%) than in the solvent hexane (3%).

3.2 Direct toxicity of natural extracts of leaves of *P. phaseoloides*
The extracts are ranked most efficient in the less active according to their toxicity (LD50) of the termite *M. bellicosus* (Table 1). Topping the list is found the hexane extract. This extract has a higher toxicity to other natural extracts of the plant, with an LD50 of the lowest 158.6 $\pm$ 0.418 mg / l. In second place is found the aqueous extract with an LD50 of 743.8 $\pm$ 0.115 mg / l. However, the alcoholic extract is less toxic to *M. bellicosus*. The LD50 is 196 times higher than that of the hexane extract.

Table 1: Toxicity of leaves extracts of *Pueraria phaseoloides* (LD50 in mg/l) on small workers of *Macrotermes bellicosus*

Hexane extract	Alcoholic extract	Aqueous extract
158.6 $\pm$ 0.4 a	3113.6 $\pm$ 0 c	743.8 $\pm$ 0.1 b

3.3 Persistence of efficiency (in days) of different leaf extracts *P. phaseoloides*
Extracts of *P. phaseoloides* remain active between 1.4 and 3.4 days (mortality rates of small workers are significantly higher than that obtained in the control at 5%) (Table 2).

Table 2: Persistence of efficacy (in days) of different leaf extracts of *Pueraria phaseoloides*

Hexane extract	Alcoholic extract	Aqueous extract
2,4 ± 0,48 b	1,4 ± 0,48 a	3,4 ± 0,48 c

The persistence of efficacy is the duration of the residual activity of an extract which generates higher mortality rates than the control at the 5%. Average of 10 replicates ± SD (N = 30). Values followed by the same letters are not significantly different at 5% level according to the Kruskal-Wallis (p <0.05, ANOVA) (H = 5.672, p = 0.023, N = 30).

3.4 Mode of action of the most toxic hexane extract of leaves of *P. phaseoloides* on the termite.

3.4.1 Consumption test

Control papers and papers treated with the extract are visited by the termite, without any difference, as shown veneers land but the termite does not consume treated papers. In contrast, the average area of paper consumed in the control is 5.58 mm^2 or 0.041 mm^2/worker (Table 3). The amount of extract ingested by the workers at the end of experience is nil however, the percentages of mortality obtained from treated workers are significantly higher than the control (35.38 % ± 17.26 of the dose of 1 mg / l to 83.52 % ± 32.94 at a dose of 10 mg / l), against 5.07 % ± 7.62 with the control in 48 hours (H = 10.352, p = 0.008, N = 50). There is therefore no correlation between mortality and consumption of the workers of the extract in the doses tested (R = 0.169, N = 50, P = 0.334 at 48 hours). The toxic effect of the hexane extract of *P. phaseoloides* is not related to its ingestion by the termite. We will specify the following tests by other ways of action of the product.

Table 3: Effect of leaves hexane extract of *Pueraria phaseoloides* on the harvesting of the workers of *Macrotermes bellicosus* (test by consumer)

Dose of hexane extract of *P. phaseoloides* (mg/l)	Cumulative area veneer (mm^2/w)	Cumulative area of paper consumed (mm^2/w)	Cumulative amount of paper Ingested (ppm/w)
0	0.96 ± 0.62 a	0.04 ± 0.06 b	0 a
1	0.43 ± 0.36 a	0 a	0 a
2	0.42 ± 0.41 a	0 a	0 a
5	1.54 ± 0.72 a	0 a	0 a
10	0.88 ± 1.01 a	0 a	0 a

Measurements taken at times corresponding to 50% mortality of workers (w). Average of 10 replicates ± SD (N = 50). Within the same column, values followed by same letters are not significantly different at 5% level according to the Kruskal-Wallis (p <0.05, ANOVA). Area Veneer (H = 3.330, p = 0.503, N = 50) - Area consumed (H = 0.942, p = 0.030, N = 50) - The amount ingested (H = 0, p = 0.000, N = 50).

3.4.2 Inhalation test

The extract does not act by ingestion; it is interesting to see if it can be toxic without being in contact with the termite. At doses of 1, 2, 5 and 10 mg / l, the inhalation of the extract does not cause mortality rates significantly higher than that obtained with the control in 24 hours and 48 hours after treatment (H = 9.452, p = 0.055, N = 50).

3.4.3 Avoidance test

Consumption experiments have shown that treated papers with the extract were not consumed. The avoidance test is confirmed. When the choice is presented, control papers and papers treated with the extract are visited by the termite as shown veneer of soil. But, no paper, treated or disposed close control, is consumed by the workers of *M. bellicosus* (Table 4). Inhibition of food intake by the extract seems to be exerted both on the consumption of treated paper than on untreated paper placed nearby.

Table 4: Effect of hexane extract of leaves of *Pueraria phaseoloides* on the harvesting of the workers of *Macrotermes bellicosus* (by avoidance test).

Dose of hexane extract of *P. phaseoloides* (mg/l)	Cumulative area veneer (mm^2/w)	Cumulative area of paper consumed (mm^2/w)
Control	0.20 ± 0.12 a	0 a
1	0.24 ± 0.17 a	0 a
Control	0.51 ± 0.58 a	0 a
2	0.48 ± 0.58 a	0 a
Control	0.41 ± 0.22 a	0 a
5	0.27 ± 0.15 a	0 a
Control	0.33 ± 0 a	0 a
10	0.33 ± 0.17 a	0 a

Measurements taken at times corresponding to 50% mortality of workers (w). Average of 10 replicates ± SD (N = 80). Within the same column, values followed by same letters are not significantly different at 5% level according to the Kruskal-Wallis (p <0.05, ANOVA). Area veneer (H = 6.682, p = 0.306, N = 80) - area consumed (H = 0, p = 0.000, N = 80) - extract quantity ingested (H = 0, p = 0.000, N = 80).

3.5 Chemical composition of the hexane extract of leaves of *P. phaseoloides*

The screening shows that the presence of flavonoids and terpenoids in the hexane extract the most active. There is an absence of alkaloids, tannins and saponins (Table 5).

Table 5: Major classes of secondary metabolites found in the solvent hexane leaves of *Pueraria phaseoloides*

Alkaloid	Flavonoide	Tanin	Terpenoid	Saponin
Dragendorff test	Sodium	Chloride ferric	Anhydride acetic	Index foam
-	+	-	+	-

4. Discussion

Our work in the laboratory confirm the observations made in Côte d'Ivoire by other authors, which describe the effective use of rows of Legume *P. phaseoloides*, perennial crop protection, against termite attack [2, 3]. On another insect, the work of Howard [9] in the United States, also demonstrate the effectiveness of this plant against the attacks of coconut leaves by immature stages of predatory *Myndus crudus*. Our work reveals the mechanism of action of this cover plant on the insect, previously described can. On the termite, the contact toxicity of the leaves hexane extract (oil), which is most effective, is not high and it does not eradicate the pest population of termites. If we compare the toxicity of the hexane extract with other total extracts of natural substances, the LD50 is 35 times less toxic than the hexane extract of neem leaves and 15 times less toxic than that obtained by contact with the hexane extract of *Combretum micranthum* leaves, tested under the same conditions [10, 11].

The mode of action, chemical composition and the residual activity of extracts could explain the low toxicity of this plant directly observed for the termite. Indeed, our results do not produce toxic effects by inhalation and ingestion of this legume by the termite. At the used doses, this plant is not repulsive because the termite does not avoid the treated papers. Our laboratory results are different from those obtained in field by Keli et *al.* [3] who described the plant as a repulsive barrier in biological fighting by plant method. But as demonstrated by the work, on termites, the repulsion of a product may depend on its concentration. The dose used in our experiment is probably not sufficient to generate a repulsive response of the extract [12].

However, our results show that the leaf oil has an anti-appetite feature on the termite. Inhibition of food intake occurs after contact or inhalation of the extract. The chemical composition of the plant could explain it. Our results indicate that leaf oil contains flavonoids and terpenoids. This result is consistent with the chemical composition described by Dirven [13], which states that the flavonoids in this plant are daidzin, daïzéine and puerarin. This plant is also rich in minerals such as cadmium and copper [13]**.** The anti-appetite feature of flavonoids and terpenoids on insects have already been mentioned by other authors [14, 15]. They also showed that mineral substances highly toxic as copper and cadmium are effective in the fight against termites. They attack the insect's digestive system and prevent it from feeding [16, 17]. Our study shows that toxic substances in *P. phaseoloides* appear to act primarily on appetite and consumption of termite. If we compare the mode of action of this legume with that of other natural substances, papaya extracts are toxic to the termite *M. bellicosus* according to at least two modes (both by contact and inhalation) and they are able transmit their toxicity in the colony, when social tasks, contact and licking, which increases the toxicity of extracts [18]. At the doses used, the extracts have no effect from 1.4 to 3.4 days after treatment. The residual activity of the extracts is low and may partly explain the low toxicity of the plant. Like many other phytophagous insects, the termite must also detoxify toxic compounds, by producing enzymes that are involved in the mechanisms of metabolic detoxification of organic pollutants [19].

5. Conclusion

Our results confirm the basis of traditional use of *P. phaseoloides* in the countryside, to anti termite control. The use of the natural oil of *P. phaseoloides*, or planting between the rows of plants, may offer an alternative to pesticides, inhibiting appetite of termites. However, this pulse does not eradicate the population of termites. Complementary analyzes on the identification of terpenoids and flavonoids active in the termite, the capabilities of formulations and the factors that can compromise their effectiveness in the field are estimated in the wild.

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