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Biopesticide Potential of *Carica Papaya* (*Caricaceae*) and Its Effects on Reproduction

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SUMMARY

The pesticidal properties of different seed extracts contained in the green fruit of the papaya tree *Carica papaya* L. (*Caricaceae*) were tested on the species of termite pest Macrotermes bellicosus. The effect of the alcoholic extract of the seeds is the most toxic and that of the aqueous extract the least (LD50 of 0.15 ± 0.0 mg/L and 23.5 ± 0.0 mg/L respectively). Contact is one of the essential factors for the effectiveness of the extract but it does not act by ingestion and it inhibits food intake by the termite. The study of toxicity on mammals indicates that the aqueous extract is non-toxic (LD50>5000 mg/Kg/bw). However, the study of the effects of the extract on male fertility shows that sperm parameters are altered 2 weeks after treatment. The use of papaya as a bioinsecticide can be justified satisfactorily but under control.

Keywords: Papaya; Pesticidal Plant; Male Fertility

Introduction

Attacks by pests such as termites on crops in Africa will increase in connection with global warming. Indeed, we have demonstrated that attacks on young rubber plants in Côte d'Ivoire by the two species of termites *Ancistrotermes* and *Microtermes* could reach 90% of the plants. The attacks were targeted on young plants under water stress (environmental factor), apart from poor planting (human factor) (Tahiri, 2022). Many chemical termiticides are used in the fight against pests with known harmful effects, in particular that of endocrine disruptor (Ano, et al. [1]). Africa has natural potential, its wealth of medicinal plants used in African pharmacopoeia. These plants can also have biopesticide properties for agriculture. Several studies have been able to demonstrate the pesticidal effects of certain plants including *Azadirachta indica* (Tahiri et al., 2011) and others such as *Cassia occidentalis* and *Thitonia diversifolia* with promising advantages as a biopesticide on cocoa and rice crops (Diby [2,3]). Even if these plants have proven effective and promising as a biopesticide in toxicity tests in the laboratory and in the field, before offering them on a large scale to producers, it is necessary to check the possible toxicity of these plants on mammals in order not to reproduce the problems of chemical pesticides. *Carica papaya* is a dietary food due to its richness in vitamins and enzymes, notably papain which is a proteolytic enzyme. The leaves, seeds and latex are used in traditional medicine.

Today, it is possible to find tablets containing only the active substance, papain. Very ripe fruits are eaten with their seeds against constipation and for deworming properties (Sastre and Breuil [4]). The fruit is used in gastric and duodenal insufficiency (Girre, et al. [5]). Latex applied in the form of hydrogel to burns, promotes healing (Boullard [6]). Traditional midwives use the root and leaves of the plant to facilitate the expulsion of the baby during difficult births (Sofowora, 1996 ; Fabert [7]). The roots and dead leaves are also used against gonorrhea and Syphilis (Boullard [6]). This study is a compilation of work by different authors (Tahiri and al., 2010; Konan [8]). The general objective is to show the effectiveness as a biopesticide of *Carica papaya*, widely consumed and used in traditional pharmacopoeias in Africa, and its possible toxicity on the fertility of mammals. More specifically, we will present in the laboratory the direct toxicity and by consumption of seed extracts on the termite pest Macrotermes bellicosus; We will present the acute toxicity of aqueous seed extracts on rats;We will present the effects of aqueous seed extracts on sperm parameters in male rats.

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Material and Methods

Animal Material

For biopesticide tests, small workers of the Termite Macrotermes bellicosus are collected at the University FHB in Cocody in Côte d'Ivoire. This species was chosen for its known impact on rice (Akpess and al., 2001) and rubber crops (Tahiri, 2022). For acute toxicity tests, they are carried out on female rats Rattus novergecus of the wistar strain, virgin, nulliparous and non-pregnant, 6 to 8 weeks old, weighing between 140 and 160 g. For the study of sperm parameters, on male between 160 and 180g. These animals underwent 5 days of acclimation with free access to water and food ad libitum, the litter was changed twice a week.

Plant Material

It is made up of seeds of the fruit of *Carica Papaya* harvested in Abidjan, Côte d'Ivoire. The samples collected in the mornings were cleaned, dried in the open air, at room temperature for 2 weeks. They were then ground into powder and stored well until use.

Methods

Preparing the Extracts

The aqueous, alcoholic and hexane extracts of *C. papaya* seed are produced according to the classic successive extraction method using solvents of different polarities. The plant organ is first exhausted by hexane, then by methanol, and finally by water. The solutions collected are evaporated by rotary evaporation in a rotavapor, to obtain total hexanic (oily), methanolic and aqueous extracts, which are dried under vacuum (Tahiri and al, 2010).

Biopesticide Tests

A 10% stock solution is prepared from each extract dried with the corresponding solvent before being tested on 1 g of fresh weight of adult small workers (PO) of the termite *M. bellicosus* (either 136 small workers), at doses of 10, 20, 50 and 100 µL per box, or at the following four contents: 1, 2, 5 and 10 mg of extract/L. The control is treated with the same doses of the corresponding solvent (Tahiri and al., 2010).

Rat Toxicity Tests

For the assessment of acute toxicity, the aqueous seed extract is obtained from a 5% stock solution with distilled water. For the evaluation of the effect of the extract on the male genital tract, the aqueous seed extract is obtained from a 5% stock solution in Nacl. The concentrations of the aqueous seed extract are administered according to the body weight (BW) of the rats (Konan [8]).

Phytochemical Screening

The detection of secondary metabolites consists of tests to characterize the large groups of chemical compounds contained in the aqueous extract. The detection of these compounds is based on the principle that they induce chemical reactions in the presence of appropriate reagents (Wagner and Bladt, 2001). These tests were carried out using the analytical techniques described in the work (Tahiri and al., 2011 ; (Konan [8])).

Biological Tests

Toxicity by Contact and by Consumption on Termites (LD50)

Two biological tests are carried out according to the protocols of Delgarde and Rouland-Lefevre [9] : the direct toxicity test makes it possible to measure the responses of termites to soil treated with the insecticide; the toxicity test by consumption makes it possible to determine whether or not the mortality of small workers results from consumption of the insecticide and specifies the importance of consumption of the product in this mortality; The mortality of the small workers was determined 24 hours after the treatments. The LD50 is calculated based on 24 hour mortality. The tests are carried out at ambient laboratory temperature of between 27 and 28 °C. Direct toxicity tests, by consumption, are carried out in a small rectangular plexiglass box measuring 95 x 65 x 20 mm in height containing 7 g of soil moistened with 2 ml of distilled water. Using a micropipette, the doses are placed either on the earth (for direct toxicity tests, or on pieces of Whatman No. 1 paper measuring 4 cm^2 for consumption tests). After depositing, the boxes are dried in the open air for 1 hour. The small workers of *M. bellicosus* are then introduced into these devices which are closed and do not allow air circulation. Each extract solution is tested at all four doses mentioned. Each dose is repeated ten times for all the tests. Each control box is treated with the corresponding solvent. The mortality of the small workers was determined 24 hours after the treatments. Each Whatman paper covered with earth veneer and that consumed (mm^2/worker) are measured every day with an ocular micrometer adapted to a magnifying glass. The quantity of extract ingested per worker per day (in ppm) is calculated (Tahiri and al., 2010).

Calculation of Mortality Percentage

The mortality percentage (PM) is calculated according to the ratio of the number of dead individuals observed to the total number of termites:

$$PM = \frac{Observed\ mortality}{Total\ number\ of\ insects} \times 100$$

Nombre total number of insectes = 50/box

Acute Toxicity in Female Rats (LD50)

The experiment was conducted according to the European OECD guideline 423. Six (6) female rats with a weight between 140-160 g were divided into 2 groups of 3 rats (a control group and a treated group). The animals were fasted the night before the experiment while having free access to water. The control batch received the single dose of 1 ml/100 g of body weight of distilled water. The treated batch received the single dose of 5000 mg/Kg body weight of aqueous extract. The change in general behavior or mortality of rats in each batch was monitored for 14 days. At the end of this period, the animals were anesthetized with ether then sacrificed (Konan [8]).

Effects on the Male Genital Tract

The study is carried out according to (Konan [8]). 20 rats male aged 9 weeks with a weight between 160 and 180g are housed in collective cages, at room temperature. The animals were fasted for 8 hours, then divided into 3 groups:

- Lot 1: the control animals received 1ml of Nacl intraperitoneally
- Lot 2, lot 3, received a dose of 6.25 mg/kg of extract by intraperitoneal injection

The rats were sacrificed two weeks after treatment (2W) and four weeks after treatment (4W).

Sperm Collection and Analysis

After treatment, the animals were sacrificed in order to carry out a sperm study based on parameters such as sperm motility, sperm morphology and sperm concentration (Konan [8]).

Collection of Sperm from the Tail of the Epididymis

The method is that described by (Konan [8]). Immediately after sacrifice, the tail of the left epididymis of each rat is removed with a pair of dissection scissors by opening the scrotum, and lacerated in the pill box containing 10 ml of Nacl 9‰ previously heated to 36 °C. Thus, the spermatozoa diffuse into the solution.

Analysis of Sperm Parameters

Sperm Motility : According to (Konan [8]), a drop of the saline solution of spermatozoa was placed between slide and coverslip and examined under a microscope (Olympus) at X 400 magnification. Motile and immobile spermatozoa were quickly counted on five random fields in a population of spermatozoa per field, then the percentage of mobile form was determined using the following formula:

$$\%\ of\ motile\,spermatozoa = \frac{Number\ of\ motile\ spermatozoa}{Total\ number\ of\ spermatozoa} \times 100$$

Percentage of Spermatozoa Abnormalities : The morphology of the spermatozoa is determined using a smear by spreading a drop of the suspension of the 0.9% saline solution between the slide and the coverslip. Stained with eosin, the slide was dried in ambient air. The stained slides are then examined under a microscope at x 400 magnification. The number of abnormal spermatozoa was expressed as a percentage of the total number (Konan [8]).

Sperm Concentration : Sperm density was determined using the Malassez cell. Thus a drop of epididymis macerate was taken and placed on the cell then covered with a coverslip. Sperm enumeration was carried out using a light microscope (Olympus CX31RBSF, Philippines) (X400)

$$N = \left(X\ x\ fd\ x\ 10^{6}\right)\ /\ 4$$

X= Number of spermatozoa counted in 5 grids of 20 small squares of the Malassez cell; fd=Dilution factor; N=Number of spermatozoa per mm3

Statistical Analyzes

The data collected during the biological tests are processed using Statistica software (2001). The box plot, boostrap estimation, non-parametric Newman-Keuls and Kruskal-Wallis tests (at the 5% threshold) and correlation tests were used. The LD50 of the biotests is calculated by Probit analysis on the basis of mortalities obtained after 24 hours on different doses.

Results

Phytochemical Screening

The aqueous extract of the seeds is richer in compound (Table 1).

Table 1: Phytochemical screening of *Carica papaya* seed extracts.

Compounds	Alcoholic seeds extract (Tahiri et *al.*, 2010)	Aqueous seed extract (Konan [8])
Polyterpene	+	+
Alkaloid	+	+
Gallic tannin	+	+
Quinone	-	+
Polyphenol	-	+

Direct Toxicity by Contact in the Laboratory on Termites

C. papaya seed extracts are toxic by contact with the small worker of the termite *M. bellicosus*. The alcoholic extract of *C. papaya* seed is the most effective and the aqueous extract the least (Table 2).

Table 2: LD50 of *Carica papaya* seed extracts on the Termite *Macrotermes Bellicosus* (direct toxicity test).

Seed extract	LD50 in 24H (mg/L)
Alcoholic	0,15 ± 0a
Hexanic	0,60 ± 0a
Aqueous	23,5 ± 0b

Toxicity by Consumption in the Laboratory on Termites

The control papers and the papers treated with the extract are visited by the termite as shown by the earth veneers. But the termite does not consume the treated papers. On the other hand, the average

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surface area of paper consumed by the control is 5 mm^2, or 0.04 mm^2/worker (Table 3). The quantity of extract ingested by the workers at the end of the experiment is zero. However, the mortality percentages obtained among the treated workers are significantly higher than that of the control (H = 9.33; p = 0.000; N = 50). There is therefore no correlation between worker mortality and consumption of the extract for the doses tested at 24 hours (R = 0.45; N = 50; p = 0.220). The toxic effect of the alcoholic extract of *C. papaya* seeds is therefore not linked to its ingestion by the termite (Table 3).

Table 3: Effect of the *Carica papaya* seed alcohol extract on the *Macrotermes bellicosus* workers collection activity (consumption test).

Dose	Cumulative plating surface (mm^2/w)	Cumulative consumed surface (mm^2/w)	Cumulative extract quantity ingested (ppm/w)
0	0,30 ± 0,23a	0,04 ± 0b	0a
1	4,50 ± 5,46ab	0a	0a
2	2,18 ± 1b	0a	0a
5	2,23 ± 1,31b	0a	0a
10	3 ± 5b	0a	0a

Note: Measurements taken at a time corresponding to 50% worker mortality. Average of 10 repetitions + standard deviation (N = 50). Within the same column, the values followed by the same letters are not significantly different at the 5% threshold according to the Kruskal-Wallis test (p < 0.05; Anova – analysis of variance). Plating surface (H = 5.890; p = 0.323; N = 50); surface area consumed (H = 0.942; p = 0.030; N = 50); quantity ingested (H = 0; p = 0.000; N = 50). w: worker

Acute Toxicity After Oral Administration of Aqueous Extracts of C. papaya Seeds (LD50)

At the end of the treatment on the rat, the results of Konan (2022) indicate that the aqueous extracts of papaya seeds at a dose of 5000 mg/kg/bw, did not reveal any toxicity parameters, namely respiratory and travel problems. Neither a loss of weight nor an excessive gain in weight was observed in the treated animals compared to the control. At the end of the 14 days of observation, no deaths were noted, likewise any change in behavior recorded in the treated animals.

Effect After Oral Administration of Aqueous Extracts of *c. Papaya* Seeds on Sperm Parameters

The Konan [8] study indicates:

Mobility: In rats sacrificed two weeks (2W) after treatment, only 6.69% of the spermatozoa collected from the caudal epididymis were mobile compared to 75.38% in the controls (p<0.0001). As for the rat sacrificed after one month (4S), the rate of mobile spermatozoa increased to 64.4%.

Morphology: The rates of abnormal spermatozoa (double head, flagellum too short, immature spermatozoa, immobile) were 93.30% (P<0.001) and 35.5% (p<0.05) respectively for the treated and sacrificed batches after 2S and 4S compared to 24.61% in untreated animals.

Concentration: The average concentration of spermatozoa taken from the epididymis is 8.66±0.46 (P<0.01) and 10.46±3.6 Million Spz/ml (P<0.05) for the treated and sacrificed after 2S and 4S against 20.66±1.4 Million spz /ml in control rats.

Discussion

The alcoholic and hexanic extracts of seeds of *Carica papaya* are the most toxic to the Termite *M. Bellicosus*. The constituents of papaya help to explain the potential insecticidal action. The alcoholic extract of papaya seeds studied here is rich in terpenoids, tannins and alkaloids. *C. papaya* has been studied for many years for its richness in secondary metabolites. The work of Fabert (2017) mentions these secondary metabolites in detail: For 100 g of seeds, we observe 660 to 760 milligrams of BTIC (benzylisothiocyanate), a glycoside, sinigrin and 2 enzymes, myrosin and carpasemin.Terpene compounds are: Carotene; Lycopene; benzylisothiocyanate or BITC which is attributed to the anti-helminthic action of C papaya. For polyphenol compounds, this is protocatechuic acid; p-coumaric acid; 5,7-dimethoxycoumarin; Caffeic acid; Kaempferol; Quercetin and Chlorogenic Acid. As for alkaloids, Papaya contains carpain which is the majority alkaloid in the leaf with a content of 1000 to 1500 parts per million; pseudocarpain and dehydrocarpains I and II. carpain which plays an important defense role against insects and herbivores and amoebicidal and hypotensive properties are attributed to it.*C. papaya* is known to be a rich source of four cysteine endopeptidases: papain, chymopapain, glycylendopeptidase, and caricaine. These enzymes represent more than 80% of the enzymatic fraction of latex. It has been isolated from the latex of *C. papaya*, other cysteine endopeptidase enzymes like Glycylendopeptidase; *Carica papaya* lipase (CPL); class II Chitinase (Fabert, 2017). Other enzymes have not yet been isolated.

The insecticidal effects of some of these constituents have been mentioned by several authors. Terpenoids have insecticidal, fungicidal, repellent and antifeedant properties (Wardell, 1987; Fortin and al., 2000). Tannins have insecticidal, larvicidal and repellent properties (Zhang et al., 1990). Alkaloids induce toxic effects on insects (Appert [2,3]). Our results also show that contact is one of the essential factors in the effectiveness of papaya extracts on termites. Toxic substances can therefore penetrate through the cuticle and stigmata of the insect. On the other hand, they do not act by ingestion and they inhibit the termite's food intake. Their use as bait against this insect is not recommended. The extracts no longer have any effect 72 to 96 hours after treatment (Tahiri and al., 2010). Like many other phytophagous insects, the termite must be able to detoxify the toxic compounds of *C. papaya* by producing enzymes which are involved in the metabolic mechanisms of detoxification of polluting organic substances (Scott, 1999).

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The study of the acute toxicity of the aqueous extract of papaya seeds in rats confirmed that this extract is non-toxic orally. This extract has an LD50 greater than 5000 mg/kg of BW, in accordance with the OECD guideline (2001). Other authors including Singh and Dubey, (2008), (Etame, et al. [7]) have also shown that the LD50 of papaya leaves was greater than 5000 mg/kg and that of papaya seed wine at a dose of 2000 mg/kg to rats did not cause any deaths and no signs of toxicity. On this basis, the extracts of this plant should therefore be recommended as a biopesticide to farmers, with simple techniques for preparing treatments. However, the in-depth study on fertility in rats indicates that the aqueous extract would cause a loss of fertility in male rats.the results of Konan [8] on the evaluation of the effects of the aqueous extract on sperm parameters, showed a very significant reduction in the percentage of motile spermatozoa in rats treated and sacrificed after only two weeks of treatment and less significant in those sacrificed after a month. Regarding the percentages of abnormal spermatozoa, they were elevated in treated rats compared to control rats.

Also, sperm density decreased significantly in the treated rats. Sperm number, motility and morphology are recognized as reproductive indices in males (Cooper, et al. [10]). Here, the aqueous extract of *C. papaya* seeds could have negative effects on male fertility. This loss of fertility would be attributed to the reduction in mobility of spermatozoa and the alteration of their morphologies, including the flagellum. After 1 month of treatment, the toxic effects of the extract on spermatozoa seem to reduce over time without, however, returning sperm quality to that of controls. Studies on molecules from medicinal plants such as lupeol from Aloe vera and Mangifera indicata, Pristimerin from the thunder vine Tripterygium wilfordii have shown inhibitory effects on the hyperactivation of the sperm flagellum without other effects (Milot [11-17]). The current results do not indicate whether the secretion of testosterone is impacted. Konan (2022) was able to verify, through a histological and morphometric study on the structure of the testicle and epididymis of treated subjects, the presence of spermatozoa and spermatogonia in the seminiferous tubules and in the caudal epididymis of treated rats. The extract does not appear to affect the testicular and epididymis structures.

Conclusion

The study confirms the insecticidal effect of papaya extracts. The toxins act by simple contact in the insect. Although natural extracts from papaya can be a solution to the use of pesticides in the fight against termites, with an LD50 indicating their non-toxicity on mammals, their use must be controlled in view of the action on the reduced fertility in males.

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