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Research Article

Phytochimical and insecticidal study of three organic extracts of *Crataeva religiosa* Forst on *Sitophilus zeamais* and *Callosobruchus maculatus*

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Abstract: The peasants use more and more the plants to protect the food products in Senegal. A phyotochimic and insecticidal study was conducted on three organic extracts (cyclohexanic, chloroformic and methanolic) of *Crataeva religiosa* on the devastating insects of maize and of niébé (*Sitophilus zeamais* and *Callosobruchus maculatus*). The data were analyzed by the procedure General Linear Model using the software Minitab 17. The results of the analysis showed that the chloroformic extract gives a better mortality rate on *Sitophilus zeamaïs* and *Callosobruchus maculatus* to the 4th day, 5th day, 6th day, and 7th day. The insect *Callosobruchus maculatus* is more sensitive to the treatment. These results are corroborated by the phyotochimic tests with the identification of molecules (alkaloids, flavanoides, tannins…) likely to be responsible for this insecticidal activity.
Key words: extracts, *Crataeva religiosa*, *Sitophilus zeamais*, *Callosobruchus maculatus*, stored food products, maize, niébé

INTRODUCTION

In sub-Saharian Africa, the losses post-harvest of cereals, before transformation, are estimated at 10-20% and approximately 4 billion dollars. These losses account for 13.5% of the total bill of the cereal production of these countries. The losses post-harvest are estimated in the 22 member countries CORAF with 10% for the cereals[1,2]. These difficulties of storage are related to several factors among which one can quote the attack of the devastating insects of maize stock and of niébé of which most frightening are *Sitophilus zeamaïs* and *Callosobruchus maculatus* respectively. Losses due to the insects strongly penalizing the commercial value of the productions and the products stored create deficits mainly filled by expensive imports of foodstuffs.

To fight against these beetles of maize and of niébé the most used method is the use of insecticides of synthesis (organophosphorus and organochlorinated…). According to Isman[3] (2006), several developing countries still resort to insecticide DDT and other persistent organic pollutants (POP). The governments even offer subsidies to the farmers to buy the pesticides. According to FAO[4], approximately 30% of the products marketed particularly in the countries of sub-Saharian Africa do not meet the international quality standards because of lack of effective method of control. The insecticides cause moreover, of the problems of availability, of storage and cost. The recourse to the pesticides of synthesis causs problems of contamination of the environment, resistance of the populations of pests, and adverse effects on the organizations not concerned[5]. The fight against insects thus becomes a pressing economic need for all the countries, whatever their degree of scientific evolution. According to[6, 7,8,9,10,11,12], the average natural ones of control constitute significant components of our systems of production. We must continue to seek them to especially diffuse them near the peasants. According to these authors, the developing countries represent the most promising outlets for the insecticides of vegetable origin. The objective of this work is to identify the various families of chemical compounds in three extracts (cyclohexanic, chloroformic and methanolic) of *Crataeva religiosa* (plant used by the buildings to protect fish smoked from the insects) and to study their insecticidal activities on *Sitophilus zeamais* and *Callosobruchus maculatus*.

MATERIAL

Plant material

Vegetable material was collected in the rural community of Keur Balla, dried in the shade at safe from the light before being conveyed in Dakar for the study. It comprises various types of drugs (barks of stem, sheets) which were crushed and used for the extractions with solvents of gradient of increasing polarities. Vegetable material consisted of sheets and barks of stem of *Crateva religiosa*.

Solvents

For the extraction, the tests of identification, the biological tests and the CCM, we used various types of solvents which are: the ethyl acetate, acetic Acid, cyclohexane, chloroform, dichmorométhane, water, n-hexane and methanol.

Reagents

For the identification and the description of the various chemical groups present in each extract, several types of reagents and witnesses were used while taking as a starting point the work by Békro *et al* [13]. For the identification and testing of different present chemical groups in each sample, several types of reagents and controls were used. For tannins, we used as control tannic acid and ferric chloride at 20% as revelator. Flavonoids and polyphenols were determined using Vitexin as control and Aluminium Chloride as reagent. The alkaloids were revealed through the reagent of Draggendorf using Cinchonine as control. The reagent of Draggendorf is prepared starting from a solution made up of 0,85g of basic nitrate of bismuth and 10g of tartaric acid in 40 ml of water (solution A) and a solution containing 16 g of KI in 40 ml of water (solution B). Extemporaneously to mix 5 ml of A, 5 ml of B, 100 ml of water and 20 g of tartaric acid.

Biological material

Animal material relates to *Sitophilus zeamais* and *Callosobruchus maculatus* obtained by mass rearing.

Methods

Harvest, drying, extraction and breeding of mass

Harvest and drying

The specimens are collected in the rural community of Keur Balla, locality located in the department of Mbour which is a subsidy of the region of Thiès ranging between the latitudes 14°02' and 15°27' Northern and Western longitudes 16°09' and 17°12'. Thus, thanks to the assistance of an old tradipratician, we collected the sheets of *Crataeva religiosa*. Vegetable material obtained was dried in the shade using the light during two weeks.

Extraction

The method used for the extractions is the maceration with solvents of gradient of increasing polarity (Cyclohexane, Administers chloroform to and Methanol). The extract obtained is concentrated using a rotary evaporator before being dried at the room temperature and safe from the light.

Mass rearing

That relates to *Sitophilus zeamais* and *Callosobruchus maculatus*. We found maize infested at the laboratory and niébé infested at the market with which one operated sorting, recovered the insects and launched the breedings. The breedings are launched in jars out of glass of 500 ml of volume approximately. Inside the jars, one put a number from 20 to 25 insects and impregnated absorbent water cotton to create the conditions of moisture necessary for a good reproduction of the insects. These jars are perforated and covered with fabric of mosquito net to make it possible the insects to breathe. The breedings are made in the shade and the room temperature (25°C approximately). At the end of 17 to 28 days, we observed emergences. The tests of insecticidal activity are carried out on insects of first generation this be-with-to say which are old between 0 and 24 hours.

Photochemical screening

The highlighting of different families of chemical compounds in *Crataeva religiosa* is made by Thin Layer Chromatography (TLC) and by staining tests and precipitation. For the identification of different chemical groups by thin layer chromatography (TLC), we relied on the course of Bassene[14] on lipid extraction. For the identification of tannins, we used as eluent a mixture of ethyl acetate, methanol and water in the proportions of 40 ml, 5 ml and 8 ml respectively. For this purpose, we used as the stationary phase glass plates covered of silica gel. The brown color of spots indicates the presence of tannin in the extracts. For flavonoids, the eluent used was a mixture of ethyl acetate and water (15%). The stationary phase was glass plates covered with cellulose. The revelation was made with Aluminum Chloride and observation under UV at 254 nm. The yellow coloring indicates the presence of flavonoids. Alongside the identification of flavonoids, may be that of the polyphenols with a UV exposure without direct use of reagents. Thus, there may be several luminescences with various colorations. To find saponins, we paid in a test tube, 10 ml of aqueous total extract. The tube was agitated for 15s and allowed to stand for 15 minutes. Height persistent foam than 1 cm indicated the presence of saponins.

Biological test

The tests relate to three extracts (cyclohexanic, chloroformic and methanolic) of *Crataeva religiosa* and two types of insects (devastating *Callosobrucus maculatus* of niébé and devastating *Sitophilus zeamais* of maize) and two speculations (niébé and maize). Each extract is tested on the two types of insect. Starting from each dry extract, we prepared five solutions of different amouns (100mg/ml, 2: 50mg/ml, 3.4 and 5). Solution 5 is obtained by taking 1g of dry extract which one dissolves in 10 ml of solvent. . Solution 4 is obtained by piping 5 ml of the solution 5 which one supplements to 10 ml with solvent. With the same process, we obtained solution 3 starting from solution 4.2 from the 3 and 1 from the 2.
The biological tests are carried out in limp of Petri of diameter 90mm. In each limps, one put 20g speculation (niébé or maize). The tests are carried out by pulverizing 500 µl of each solution in limp of Petri thanks to a pipette Pasteur. The test is repeated five times for *Sitophilus zeamais* and four times for *Callosobruchus maculatus*. The whole is then left with the free air during 20 mn to allow the evaporation of solvent. The insects are introduced thereafter into each limp. On the whole, we used 1215 experimental units to evaluate the toxicity of all the extracts on the insects is 135 units per extract (135× 9 = 1215). The insects are introduced thereafter into each limp. The dead insects are sorted and recovered using aluminium grip. The number of died, alive and emerged insects are then counted. The formula of Aboth: Mc = (Mo-MT)/(100-MT) *100; (with Mc: calculated mortality, Mo: mortality observed and MT: mortality in the pilot batches) is used to correct mortality observed.

Statistical analysis

For the data of the biological tests with the extracts of the plant, the measured variables are the number of died insects, the number of surviving insects and the number of emerged insects. Calculated mortality was obtained by applying the formula of Aboth (1925): Mc = (Mo-MT)/(100-MT) *100; (where Mo = mortality in the treated batches, MT = mortality in the witness and Mc = calculated mortality). The variables many died insects, number of the surviving insects and number of the emerged insects are subjected to a variance analysis, model fixed with three factors (extracted, amounts and time). Variable mortality rate underwent a transformation arcsin (X = mortality rate, N = size of the population; n= 1999) in order to standardize the population and to stabilize the variance. The method General Linear Model in Minitab 17 was used for the statistical analysis of the collected data. The variables many surviving insects and many insects emerged as for them underwent a transformation square root in order to standardize the population and to stabilize the varian. The curves and the tables are used to have the result of the analysis.

RESULTS

Results of the extractions

From 64,154 g of powder of the plant (*Crataeva religiosa*), one carried out three extracts cyclohexanic, chloroformic and methanolic. The results of the extractions are confined in the following table:

Table 1: Results of extractions

Extracts	Aspect	Mass (g)	Output
Cyclohexane	Powder	2.8	1. 436%
Chloroform	Powder	2.6	1. 238%
Methanol	Pasty	6.742	10.51%

For the extractions, one notes the best output on the level of the methanolic extract. The cyclohexanic and chloroformic extracts present the same aspect (powder), whereas the methanolic extract is pasty.

Phyotochimic study

Table 2: Results of the phytochimic tests

Extracts	Alkaloids	flavonoïdes	Polyphenols	Tannins	Saponosides
Cyclohexanic	+	-	+	-	-
Chloroformic	+	+	+	+	-
Methanolic	-	+	+	+	-
Aqueous	-	-	-	-	+

+: presence, -: absence

The table of the phytochimic tests shows the presence of alkaloids in the cyclohexanic and chloroformic extracts of the plant. The flavonoides are present that in the methanolic extract. The three extracts cyclohexanic, choroformic and methanolic contain all of them polyphenols. As regards tannins, we identified them in chloroformic and methanolic extracts. The search for saponosides is positive in the aqueous extract of *Crataeva religiosa*.

Biological tests

Analysis of the variance corresponding to the insecticidal effect of the extracts of *Crataeva religiosa* watch which mortality has a highly significant variation according to time, the extracts and the insects (P<0,001). In the same way, the interactions time-extracts are significant (P<0,05). The factor amount is not significant (P>0,05). The analysis also shows that mortality has a significant variation according to the interactions time-extract-insects (P<0,05). What shows that the insecticidal effect depends on time, the extracts and the insects.

Table 3: **Result of variance analysis of observed parameters**

Source of variation	Mortality			Emergence		
	DL	F	P	DL	F	P
Time	1	15, 67	0, 000	1	1, 80	0, 181
Doses	4	0, 24	0, 913	4	4, 65	0, 001
Extracts	2	11, 73	0, 000	2	47, 15	0, 000
Insects	1	23, 07	0, 000	1	114, 16	0, 000
Time doses	4	0, 16	0, 960	4	2, 89	0, 022
Time extracts	2	5, 90	0, 003	2	44, 52	0, 000
Time insects	1	3, 69	0, 055	1	138, 16	0, 000
Doses extracts	8	0, 29	0, 970	8	3, 47	0, 001
Doses insects	4	0, 57	0, 687	4	3, 15	0, 014
Extracts insects	2	0, 54	0, 583	2	44, 88	0, 000
Time doses extracts	8	0, 42	0, 910	8	3, 40	0, 001
Time doses insects	4	0, 38	0, 821	4	1, 58	0, 178
Time extracts insects	2	3, 06	0, 047	8	3, 40	0, 001
Doses extracts insects	8	0, 57	0, 804			
Time doses extracts insects	8	0, 47	0, 879			
Error	945			375		
Total	1259			499		

The figures show the evolution of the treatment according to time, the amounts, the extracts and the insects. The extract with chloroform (EH) is effective on *Callosobruchus maculatus* and *Sitophilus zeamaïs* while extracts with Cyclohexane (EC) and methanolic (EM) are effective only on Callosobruchus maculatus. The curves also show that the extract with Chloroform gives a better mortality rate and that *Callosobruchus maculatus* is more sensitive to the treatments the strong amount (50mg/mL). The curve of mortality according to time shows that at the dates 4th day, 5th day, 6th day, and 7th day mortality is higher.

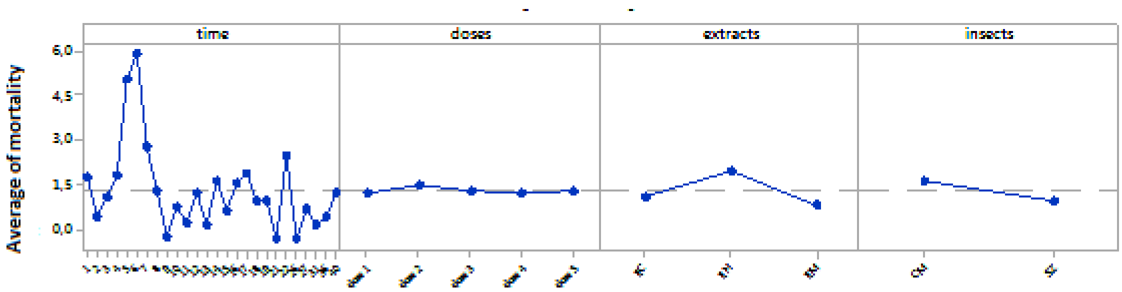

Figure 1: Curve of mortality in function of doses, extracts, insects and time. EC: cyclohexane extract. EH: chloroform extract. EM: methanolic extract. dose 1: 6. 25g/l. dose 2: 12. 5g/l. dose 3: 25g/l. dose 4: 50g/l. dose 5: 100g/l.

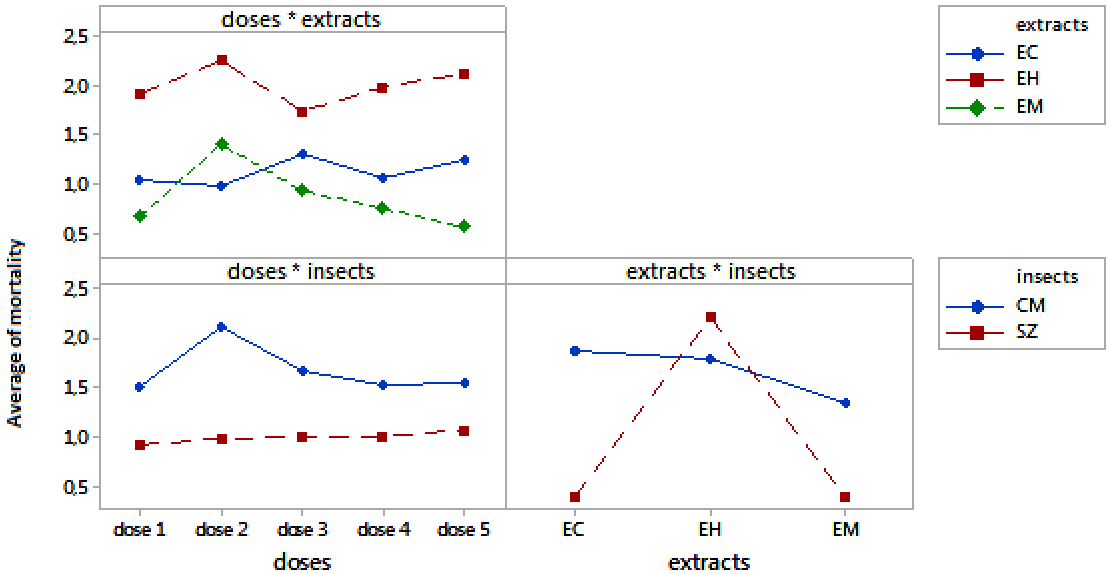

Figure 2: Interaction between extracts, doses and insects of *Crataeva religiosa*, case of mortality

The figures give the shape of the curve of the insects emerged according to time, the amounts applied, the extracts and the insects. The methanolic extract ensures a better protection of the medium on *Sitophilus zeamais* and long-term (33th day, 39th day, 44th day).

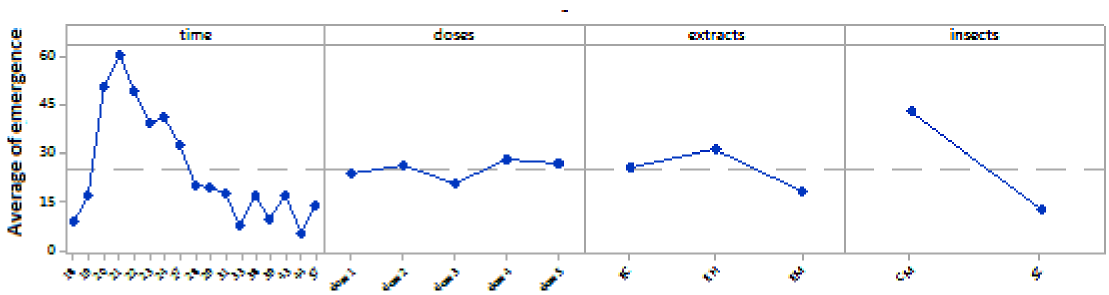

Figure 3: Curve of insects emerged in function of extracts, doses, insects and times of *Crateva religiosa*

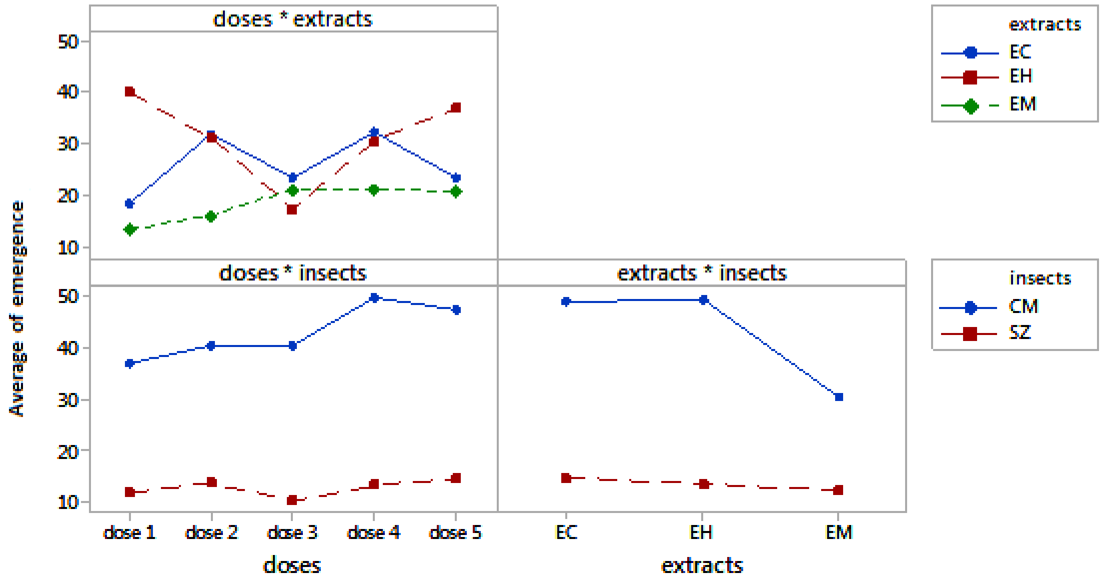

Figure 4: Interaction between extracts, doses and insects of *Crataeva religiosa*, case of the emerged insects

DISCUSSION

The statistical analysis shows that the chloroformic extract gives a better mortality rate on *Sitophilus zeamais* and *Callosobruchus maculatus*. In addition, the phytochimic study of the chloroformic extract contains flavonoides, tannins, and alkaloids. In the same way, it was shown that the species *Crataeva religiosa* contains a saponoside, tannin, terpenic compounds and nine flavonoides among which most abundant are in the order: rutin, quercetin and the isoquercétine[15]. The polyphenols ubiquitaires in nature, cause a disturbance of the natural motricity of the insect. This one can be fast: as of the first day for quercetin, or later, the fourth day, for the narangine, syringaldehyde or vanillic acid. It is accompanied in certain case (cafeic and ferulic acids, vanillin, luteoline 7-glucoside) by an effect knock down.

After eight days the insects are in a coma or dead state. At the end of eight days all the insects are in a state of coma or dead. The toxicity of polyphenols is positively correlated with the attractive power of compound[6].

Tannins present a toxic direct effect for certain species of insects[16]. Tannins influence on the growth, the development and the fertility of several devastating insects[17]. The reduced growth caused by tannins has major drawbacks for the insect, with a lower number of eggs and more a small of eggs, which would affect the survival and the health of the individuals of the generation subsequent[18]. Thus the aromatic plants and their allelochimic molecules carry on a double activity:
- on adults by rapid toxic inhalation (monoterpenes) on the one hand and on the other hand action which contributes to the insecticidal activity of the aromatic plant of a lower intensity but is exercised in the period (polyphenols);
- on the different phases of the reproductive cycle: inhibition of fertility and larvicidal and ovocide activity at neonatal and later stages.

The alkaloids have repulsive properties or anti appetizing with regard to the devastating insects[19]. Several studies showed that species of the family of Capparidaceae showed the insecticidal effect of the organic extracts on the devastating insects of stock of harvest. Among this work, it can quote those of Gueye[20,21] which showed the insecticidal activity of *Boscia senegalensis* on *Caryedon serratus* (groundnut beetle). Many work also showed that the organic extracts of plant give insecticidal effects on the devastating insects of stored food products. The toxicity of the extracts with organic solvents of *Afrostyrax lepidophilus*, *Trichilia gilgiana*, *Drypetes gossweileri* and *Zanha golungensis* with regard to *Sitophilus zeamaïs*, *Tribolium castaneum* and *Rhyzopertha dominica* is shown by work of Aba Toumnou[22] (2013) in its thesis.

CONCLUSION

Insecticidal tests of the three extracts (cyclohexanic, chloroformic and methanolic) of *Crataeva religiosa* watch which the chloroformic extract gives a better mortality rate on *Sitophilus zeamaïs* and *Callosobruchus maculatus*. The tests also showed that *Callosobruchus maculatus* is more sensitive to the extracts of the plant. This result is confirmed by the phytochimic study. This extract will be subjected to a fractionation and to purification in order to isolate the active (s) principle (s).

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