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PHYTOCHIMICAL TESTS AND LARVICIDE ACTIVITY OF THREE ORGANIC EXTRACTS FROM THE SEEDS OF INDIGOFERA PILOSA ON LARVAE OF MOSQUITOS

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SUMMARY

In the search for alternative method beside the use of dangerous insecticides for health and the environment, the vegetable kingdom offers many possibilities. Work is carried out in this direction and showed an effectiveness of the extracts of plants. Our results indicate that the chloroformic contents present a better larvicide activity than other extracts. In addition, the analysis by chromatography (CCM) of the three extracts of seeds showed their wealth of flavonic compounds, alkaloids and tannins thus suggesting a correlation between the larvicide activity and the contents in secondary metabolites of seeds.

KEYWORDS: *Indigofera pilosa*, stems, chloroformic extract, extraction, ccm, larvicide activity.

1. INTRODUCTION

The mosquitos were always regarded as source of nuisance for the man, mainly because of the fact that they can be disease vectors.[1] In Senegal, paludism, parasitic disease of hydrous origin, continue to pose public health problems. In the anti-mosquito fight, the active matters as insecticides used belong to the family of organophosphorus, pyrethrinoid and carbamates of synthesis. However although they are very effective on the mosquitos, they present several disadvantages. Indeed, besides their costalso the significant accumulation of active matters in the treated, watery and terrestrial ecosystems poses a real problem of pollution.[2]

In the search for alternative method, the vegetable kingdom offers many possibilities. Work is carried out in this direction and showed an effectiveness of the extracts of plants. Indeed the plants constitute a source of natural substances which present a great potential of application against the insects and other parasites of plants and animal kingdom.[3]

The objective of this work is to identify the families of chemical compounds present in seeds of *Indigofera pilosa* in order to explain the larvicidal activities.

2. MATERIAL AND METHODS

2.1. Material

2.1.1. Vegetable material

Vegetable equipment is composed of seeds of *Indigofera pilosa*. The plant was collected in the zone of Niayes (Senegal) in April 2015.

2.1.2. Animal material

Animal material consists of larvae of mosquitos.

2.2. Methods

2.2.1. Preparation and preservation of vegetable samples

After having collected the plant, we separated the various parts of the plant. Thus we have the seeds of the plant dried with at room temperature of the laboratory.

Following three weeks of drying, we crushed the samples of the plant through an electric crusher.

2.2.2. Collection and preservation of animal material

The larvae of mosquito are collected around the Channel IV of Fass (Dakar, Senegal). For the occasion, a surmounted pot of a long sleeve is introduced into water while inclining its edge of with 45°, under the effect of the forces of tension, the surface layer of water is thus attracted as well as the specimens which survive it. The larvae are preserved in jars of 1L filled at the three

quarter with distilled water. In the laboratory, we used the larvae of mosquitos of stage 3 and 4.

2.2.3. Extraction

The technique of practiced extraction is maceration. Indeed, the samples are impregnated in solvents (1g/10mL) of increasing polarity during 72 hours. The solvents are in the order: cyclohexane, chloroform, butanol, methanol and distilled water.

The extracts obtained are concentrated using a rotary evaporator during about 30 to 45min at temperatures around boiling points of solvents according to the extract. Thereafter, the concentrated extracts are dried screened from light at room temperature from two to six days.

2.2.4. Identification of the chemical groups

2.2.4.1. CCM of alkaloids

For the identification of alkaloids we used silica gel like stationary phase;eluant is a mixture of chloroform and diethylamine (45V/5V); the witness used is Cinchonin. The revelation is made in Draggendorf. Orange red coloring would indicate the presence of alkaloid in the extracts. The development is carried out with at room temperature and the atmospheric pressure.

2.2.4.2. CCM of tannins

The acetate mixture of ethyl/methanol/water in proportions (40V/8V/5V) is used like eluant. Plates out of glass covered with silica gel are used like stationary phase. The revelation is made by a ferric chloride solution after drying. Coloring chestnut of the spots indicate the presence of tannins in the extracts. The chromatography is carried out with the room temperature and the atmospheric pressure.

2.2.4.3. CCM of the flavonoids

Éluant used is a mixture of ethyl acetateand water with 15%. The silica gel is used as stationary phase. The revelation is made with aluminum chloride and the observation under UV with 254 Nm. Yellow coloring would indicate the presence of flavonoids.

2.2.4.4. Identification of the saponosides

In test tubes, one poured 10ml aqueous total extract. Each tube is agitated vigorously during 15 seconds, then left at rest during 15 minutes. A height of persistent foam, higher than 1 centimeter would indicate the presence of Saponosides.

2.2.5. Biological tests

The larvicide activity was undertaken according to the method of the tests of sensitivity standardized by the World Health Organization, adopted to test the sensitivity of the larvae, with respect to insecticides used in fight campaigns.[7,8]

The experimental protocol is the following one:
Starting from each dry extract, we prepared five solutions with amounts of increasing concentrations (100mg/mL, 50mg/mL, 25mg/mL, 12.5mg/mL and 6.25mg/mL). 10 larvae of stage 3 and 4 were taken using a flexible grip and were put in goblets of 5 cm diameter, containing each one 40 ml of water. The same number of larvae was placed in a pilot goblet containing 50 ml of water. Four repetitions were carried out for each dilution like the witness. The formula of Abbott is then used to correct mortalities observed.

2.3. Statistical analysis

The studied factors are time, the number of died insects, the amount and the nature of the extracts like their interaction. The method General Linear Model in Minitab 17 was used for the statistical analysis of the collected data.

3. RESULTS

3.1. Phytochemical Tests

The results of phytochemical tests are consigned in the following table:

Table 1: Results of phytochemical tests on the seeds of *Indigofera pilosa*

Part of the plant	Extract	Flavonoids	Alkaloids	Tannin	Saponosids
Seeds	cyclohexane	+	+	-	-
	chloroform	-	+	-	-
	butanol	+	+	-	-
	methanol	+	+	+	-
	aqueous	-	-	-	+

+ : presence; - : absence.

3.2. Extraction

The results of the extractions are gathered in the following table:

Table 2: Results of the extractions of the seeds of *Indigofera pilosa*

Part of the plant	Extracted	Mass initial (g)	Mass of the extract (g)	Output (%)	Aspect of the extract	Color of the extract
Seeds	cyclohexane	59.00	1.11	1.88	Pasty	green clear
	chloroform	58.26	1.11	1.00	Pasty	green bed
	butanol	48.43	0.17	0.30	Gelatinous	yellow clear
	methanol	28.00	1.10	3.93	Powder	orange red

3.3. Identification of the chemical groups

The results of the tests of identification revealed by thin layer chromatography (CCM), of the various chemical groups announced by phytochemical tests on the extracts, are photographed and presented hereafter:



Photograph 1: CCM of alkaloids Photograph 2: CCM of tannins Photograph 3: CCM of the flavonoids

Eluant: acetic acid with 15% in water acetate of ethyl/methanol/water (40v/8v/5v) Acetate of ethyl/water (15%)

3.4. Biological tests

Table 3: Results of treatments with the extracts of the stems of *Indigofera pilosa*

Source of variation	Mortality		
	DL	F	P
amounts	3	0,29	0,830
time	3	134.06	0.000
extracts	2	44.24	0.000
amounts-time	9	0.83	0.594
amount-extracts	6	0.76	0.604
extracted-times	6	40.85	0.000
amount-time-extract	18	0.61	0.891
error	144		
total	191		

DL: freedomdegree F: Frequency P: Probability.

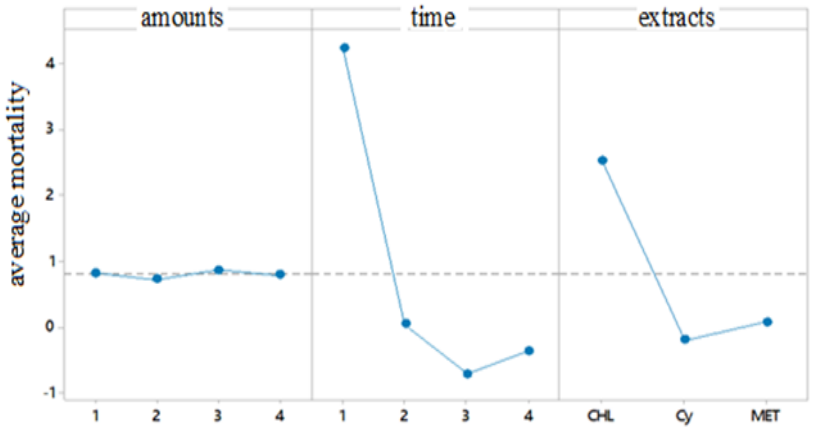

Figure 1: Curve of mortality according to the amounts, times and extracts of stem of *Indigofera pilosa* on the larvae of mosquitos. CHL: chloroformic extract, Cy: cyclohéxanique extract, MET: méthanoliques extract

4. DISCUSSION

The outputs of the extraction obtained are more important for methanol (3.93%). Methanol more polar than the others made up organics being used like solvent, one can think that the seeds of *Indigofera pilosa* contain polar compounds.[6] According to table 1, phytochemical studies showed that most extracts of various parts of the plants contain secondary metabolites. We indeed notes the presence of flavonoids in the cyclohexanique extracts, butanolic and methanolic, of alkaloids in all the extracts. Only the methanolic extracts contain tannins. Also the aqueous extracts of seeds of Indigofera contain saponosides. These results were indeed corroborated by the tests of identifications revealed by thin layer chromatography (CCM). The presence of these metabolites would prove in a way their use in fine therapeutic, ichtyotoxic and larvicidal activities. Indeed some flavonoids contain repulsive compounds such as aldehydes of flavonoids used as repulsive against the harmful insects (flies, cockroaches, plant louses, aleurodes, mosquitos, ticks, chips…).[7] According to Sylvie Morel[8] the role of the flavonoids in the interactionsin practice the rotinoïdes and in particular the rotenone were largely studied for their insecticidal activity. The degueline and the tephrosine (rotinoids) seem good larvicides against *Aedes aegypti*,[9] Lastly, some pterocarpanes have insecticidal properties against. The seeds of *Indigofera pilosa* contain alkaloids, which consist of a large number of chemical compounds which have almost both a toxicological and pharmacological activities.[11; 12; 13]

Besides their antifungal and antiviral activity[14], tannins present a toxic direct effect for certain species of insects [15]. And our study gave results which seem to attest the presence of this compounds in seeds.

The analysis of the variance corresponds to the effect of the extracts of seeds on the larvae of mosquitos. Table 3 shows that mortality with a very significant variation according to time and extracts ($P < 0.001$). Also the interactions time-extracts are very significant ($P < 0.05$). On the other hand the factors amounts, amount-times and amount-extracts are not significant ($P > 0.05$). Thus the larvicide effect depends on time extract.

With regard to time, twenty-four hours prove to be sufficient so that the extract act effectively on the larvae of mosquitos. The most effective extract is the chloroformic extract. Figures 1 and 2 reveal all that.

5. CONCLUSION

Through this work, we practiced a simple method which allowing to estimate the larvicide activity of the seed extracts of *Indigofera pilosa*. Studies more pushed by using high technologies such as (HPLC, NMR…) for the identification of the structures which are in our extracts could allow to obtain accessible biocides for our populations and without danger to the environment. Following these results, it would be advisable to make more thorough studies to find compounds responsible to larvicide.

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