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# Evaluation of *Ricinus communis* Extracts as a Biopesticide for *Nisotra gemella* Control

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Author MEM wrote the first draft of the manuscript, managed the analyses of the study and the literature searches. Author LPL designed the study, performed the statistical analysis and wrote the protocol. Both authors read and approved the final manuscript.*

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## ABSTRACT

The efficacy of the extracts of leaves of castor *(Ricinus communis)* on flea beetle *(Nisotra gemella)*, ravaging sorrel *(Hibiscus sabdariffa)*, was performed in the laboratory of Phytochemistry of the Faculty of Sciences (Loyola University of Congo). The insects were captured on the leaves of *Hibiscus sabdariffa* in the field using plastic jars.
Toxicity tests were conducted in laboratory conditions in Petri dishes at a temperature of 28 ± 1℃. The results of the preliminary toxicity test of the total extracts showed a mortality of 100% for the petroleum ether extracts, 31.33% for the ethanolic extract, and 0% for the total aqueous extract, after 24 hours of exposure. The petroleum ether extracts was chosen for further investigations.
Different concentrations of the total petroleum ether extract were applied on filter paper (1%: 0.53 µl / cm²; 0.1%: 0.05 µl / cm²; 0.05%: 0.03 µl / cm²) caused 100% mortality for the dose of 0.53 µl / cm² in the interval of 9 and 10 hours; 0.05 µl / cm² caused 100% mortality after 15 -16 hours. An interval of 19 to 20 hours was necessary to cause 100 % mortality using the concentration of 0.03 µl / cm². No mortality was recorded in control plates (petroleum ether).

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The same doses were used to evaluate the repellent effect. It was observed a low insect activity with an average rate of 39.5%. The thin layer chromatography enabled the identification of 10 spots (petroleum ether and ethyl acetate/ 5:1) and 8 spots (Petroleum ether and ethyl acetate/ 9:1).

*Keywords: Ricinus communis; extract petroleum; Hibiscus sabdariffa; Nisotra gemella; biopesticide.*

## 1. INTRODUCTION

*Hibiscus sabdariffa* commonly called roselle or sorrel of Guinea, is a popular vegetable for its leaves and chalices that go into the preparation of sauces and other culinary dishes. In the Democratic Republic of Congo (DRC), leaves are used as vegetables, but also in traditional medicine to treat anemia and fever. The green leaves can be eaten raw or cooked [1,2]. In addition, production of *Hibiscus sabdariffa* is an important source of income for growers. A study conducted by Action Against Hunger has ranked *Hibiscus sabdariffa* in third place of leafy vegetables grown in Kinshasa after amaranth and sweet potato leaves with an average revenue of $ 245 / acre [3].

However, this vegetable is facing attacks from beetles (*Nisotra gemella*) which pierce the leaves of young plants, causing a reduction in leaf area and yield reductions. According PIP [4], flea beetles can cause losses up to 70% when installed in the field. To solve this problem, most gardeners use as means of control, chemical pesticides. Unfortunately, access to these pesticides is expensive, and their use has adverse effects on the environment, human health and promotes the development of resistant strains. The awareness of the environmental cost of the increased use of chemical pesticides and fears of consumers of the danger which may be the residues of these pesticides on human health are born a growing hope for other alternatives to fight [5].

Alternative methods such as biopectide control are viable options to be used in agriculture. Aouinty [6] showed more than 2 000 plant species with insecticidal activity. Ndomo evaluated the insecticidal properties of leaves of *Callistemon viminalis* (Myrtaceae) against adults of *Acanthoscelides obtectus* (Say) (Coleoptera; Bruchidae) ravaging the bean [7]. The results showed that the essential oil of this plant is a potent insecticide against these insects. Another study by Benayad reveals that *Melia suaveolens* can have a toxic effect on *Sitophilus oryzae* causing a mortality of 100% for a low dose [8].

In the same vein, considering the above constraints due to the use of chemical pesticides in the fight against crop pests, we are facing the local flora which abounds in many species deemed insecticides and / or repellents to fight against *Nisotra gemella* (NTG) which is an important pest of kenaf (*Hibiscus* spp.) (Fig. 1) [9].

*Ricinus communis* (RCM) is a species of perennial flowering plant in the spurge family, *Euphorbiaceae* (Fig. 2). It is the sole species in the monotypic genus, *Ricinus*, and subtribe, *Ricininae*. The plant is known for its medicinal properties. Castor oil has many uses in medicine and other applications. An alcoholic extract of the leaf was shown, in lab rats, to protect the liver from damage from certain poisons [10-12]. Methanolic extracts of the leaves of RCM were used in antimicrobial testing against eight pathogenic bacteria in rats and showed antimicrobial properties. The pericarp of RCM showed central nervous system effects in mice at low doses. At high doses mice quickly died. A water extract of the root bark showed analgesic activity in rats [13]. Antihistamine and anti-inflammatory properties were found in ethanolic extract of RCM root bark [14].

The objective of this research was to evaluate the efficacy *in-vitro* of RCM extracts as a biopesticide for NTG control.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Plant Material and Insects

This study was conducted in the Laboratory of Phytochemistry, department of Chemistry (ULC) in Kinshasa, Democratic Republic of Congo from April to June 2015.

RCM were collected in April 2015 in Kimwenza (Kinshasa), the municipality of Mont Ngafula, Bianda neighborhood, then dried in the laboratory of Phytochemistry at an ambient temperature of 28 ± 1℃ under shade.

**Fig. 1. *Ricinus communis***

**Fig. 2. *Nisotra gamella***

*Source : http://www.beetle-diversity.com/node/6786*

NTG adults were used as animal material. Insects subject to toxicity testing were captured near the experimental farm of the faculty of agronomy. They were transported to the laboratory in plastic jars, and the identification was made using literature [9].

## 2.2 Solvent Extraction

Three solvents of different polarity were used for extraction: petroleum ether (PLE), ethanol (ENL) and distilled water ($H_20$). For maceration and extraction, we followed the method described by Luhata [15]. The leaves were dried for 2 weeks in the laboratory of Phytochemistry at room temperature ±28℃ and under shade to avoid decomposition of certain chemical groups by sunlight. They were crushed using a brand electric grinder GEEPAS model GSB2031. 100 g powder quantities were respectively soaked in 600 ml of ethanol, 600 ml of petroleum ether and 600 mL of distilled water. Maceration took place in a 1000 mL Erlenmeyer closed to prevent evaporation of the solvent. To speed the extraction process, the Erlenmeyer was stirred for 12 hours with a brand agitator BECTON DICKINSON Model 54, then left to stand for 24 hours. The extracts were then filtered using a funnel and cotton wool to collect the total extracts.

## 2.3 Estimated Quantity of the Residue

In order to give a logical meaning to the quantities of soluble plant materials in the various extracts, we let evaporate, under the shade, the solvents. Residues obtained were weighed to be quantified. The different weights obtained were expressed in grams. With the mass obtained for each sample, we were able to calculate the extraction rate according to the formula:

$$\frac{Quantity\ of\ Residue(g)}{Quantity\ of\ the\ dry\ leaves(g)} \times 100$$

## 2.4 Preparation of Formulations

$$\%_1\, x\, V_1 = \%_2\, x\, V_2$$

$\%_1$: The stock solution/Initial concentration
$V_1$: Initial volume
$\%_2$: Final concentration
$V_2$: Final Volume

To prepare a concentrated solution to 1%, 1 ml of the 10% solution was diluted in 9 ml of petroleum ether; for a 0.1% stock solution, 1 ml of the 1% solution was diluted in 9 ml of petroleum ether. And finally, 5 ml of 0.1% were mixed with 5 ml of petroleum ether to give a 0.05% concentrated solution.

### 2.4.1 Preliminary test

To determine the most active extract on flea beetle (*Nisotra gamella*), a preliminary test of various extracts of *Ricinus communis* was performed. Bioassays were carried out in the laboratory, with an average temperature of 28 ± 1℃. 1 ml of each extract solvent (maximum dose) was uniformly spread on a filter paper disk (56,72 cm²) of surface and a dose of 18 µl / cm²). The filter paper was dried in air for 15 minutes to allow the solvent to evaporate before putting into Petri dish. A population of 20 insects was introduced into Petri dishes and immediately closed. Three repetitions were performed and the

counting of dead insects was performed after 24 hours. Note that the controls, consisting of solvent only, received the same treatment.

### 2.4.2 Direct toxicity test

3 ml of each of the prepared solutions (as mentioned in 2.7) were spread evenly on filter paper discs (Whatman No. 1) of 8.5 cm diameter laid in Petri dishes in glass of the same diameter. These washers were left at room temperature for 15 minutes to allow the complete evaporation of the dilution solvent. For the control, the filter papers were treated only with petroleum ether (3 ml). A lot of 20 non-sexed adult insects freshly harvested in the garden was placed in each Petri dish containing a washer treated; then the plates were immediately closed.

Three repetitions were performed for each concentration and control. The resultants were obtained after 24 hours. Note that the same methodology was used to observe the evolution of the dead versus time.

### 2.4.3 Avoidance test

The avoidance test on adult NTG was evaluated using the method of the preferential zone on filter paper. The filter paper discs 8.5 cm in diameter used were cut into two equal portions each having 28.36 cm² of surface. Three doses of the total petroleum ether extract of RCM were used (0.53; 0.05 and 0.03 µl / $cm^2$) by dilution with petroleum ether.

1.5 mL of each solution was uniformly spread on a half of the disc while the other half received only 1.5 mL of petroleum ether. After fifteen minutes, the time required for complete evaporation of the dilution solvent, the two halves discs were reunited by means of an adhesive strip. The paper disc filter thus constituted was placed in a Petri dish and a set of 20 adult insects were placed in the center of each disc. Three replicates were conducted for each dose. After two hours, the number of insects on the portion of filter paper treated with total extract of RCM petroleum ether and the number of those present on the control were identified. The percentage of avoidance was calculated using the following formula:

$$PR = \frac{Nc - Nt}{Nc + Nt} \times 100$$

PR: repulsion percentage

Nc: Part treated with the extract of total
Nt: Part treated with the solvent

The average percentage of repulsion for the total extract of petroleum ether of RCM was calculated and assigned as ranked by McDonald et al. [16] (Table 1).

**Table 1. Percentage of repulsion ranking**

| Class | Percentage of avoidance (%) | Activity |
|---|---|---|
| 0 | <0,1 | No activity |
| I | 0,1-20 | Very weak activity |
| II | 20,1-40 | Weak activity |
| III | 40,1-60 | Average activity |
| IV | 60,1-80 | Strong activity |
| V | 80,1-100 | Very strong activity |

### 2.4.4 Treatments

For the direct toxicity test on filter paper, we used the following treatments:

- $T_0$: 3 mL of petroleum ether;
- $T_1$: 3 mL of the total extract of the petroleum ether on RCM concentrated to 1% (or a dose of 0.53 µ/$cm^2$);
- $T_2$: 3 mL of the total extract of the petroleum ether extract on RCM concentrated 0.1% (or a dose of 0,05µl /$cm^2$);
- $T_3$: 3 mL of the total extract of the petroleum ether extract on RCM concentrated to 0.05% (equivalent to a dose of 0.03 µl /$cm^2$).

For the avoidance test, the treatments used were:

- $T_1$: 1.5 ml of the total extract of the petroleum ether extract on RCM concentrated to 1% (or a dose of 0.53 µl /$cm^2$) + 1.5 mLof petroleum ether;
- $T_2$: 1.5 ml of the total extract of the petroleum ether extract on RCM concentrated to 0.1% (or a dose of 0.05 µl /$cm^2$) + 1.5 mL of petroleum ether;
- $T_3$: 1.5 ml of the total extract of the petroleum ether extract on RCM concentrated to 0.05% (equivalent to a dose of 0.03 µl /$cm^2$) + 1.5 mL of petroleum ether.

### 2.4.5 Thin layer chromatography (TLC)

The chromatographic plates were introduced into the prepared solvent systems contained in

beakers. The first plate was introduced into the system petroleum ether and ethyl acetate (9:1), and the second plate in the second solvent system (5:1). To identify the different groups of substances, the plates were soaked in a solution of vanillin (in concentrated sulfuric acid and methanol) and then heated on a hot plate.

## 3. RESULTS

### 3.1 Extraction

The figure below shows the rate of extraction of various extracts from 100 g dry leaves:

Ethanol as solvent extracted more plant material than the two others. The amount of plant material extracted in 100 g of dry matter is 8.6 g (8.6%) compared with EPR and EAR extracts of which the respective extraction rates were 2.8% and 3, 6%.

From Table 2, we observe that on the three repetitions, only the total petroleum ether extracts and total ethanol extract of RCM caused the death of insects with 100% for EPR and 31. 33% for ERA. EAR and solvents have exhibited no activity with a mortality rate of 0%.

Table 3 shows that the mortality rate for the control negative (0%) and 100% for the corresponding three treatments at three different concentrations: 0.1%, 0.1%, 0.05%.

From Table 4, the total elimination of the population is between 9 and 10 hours for T1 (1%); between 15 and 16 hours to T2 (0.1%); and between 19 and 20 hours to T3 (0.05%).

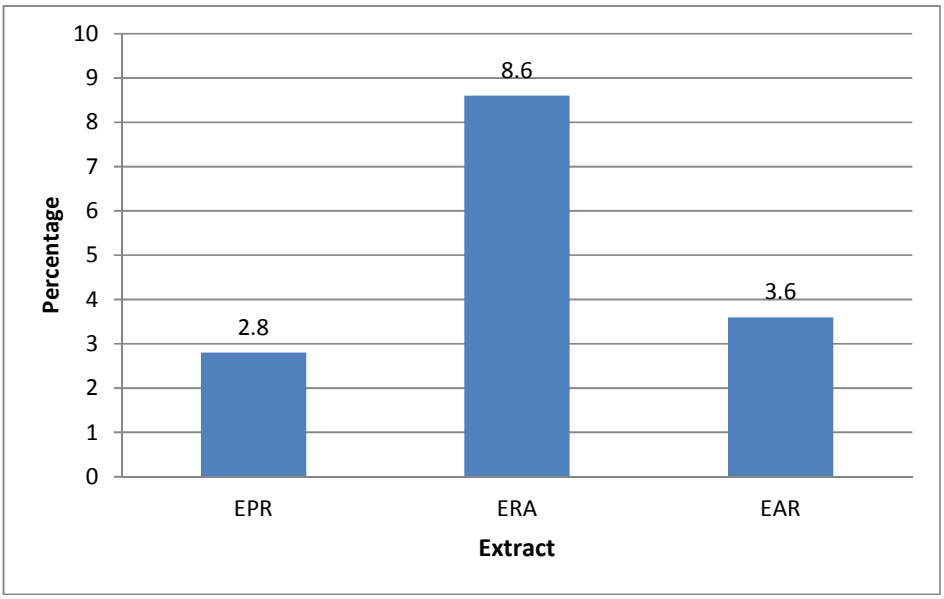


**Fig. 3. Different solvents extraction rate**

*(With EPR: total petroleum ether extract; ERA: total ethanol extract and EAR: total aqueous extract)*

**Table 2. Direct toxicity testing of different of leaves extracts**

| | ETH | | FTE | | EPR | | ERA | | EAR | |
|---|---|---|---|---|---|---|---|---|---|---|
| | M | V | M | V | M | V | M | V | M | V |
| $R_1$ | 0 | 20 | 0 | 20 | 20 | 0 | 5 | 15 | 0 | 20 |
| $R_2$ | 0 | 20 | 0 | 20 | 20 | 0 | 7 | 13 | 0 | 20 |
| $R_3$ | 0 | 20 | 0 | 20 | 20 | 0 | 7 | 13 | 0 | 20 |
| Total | 0 | 60 | 0 | 60 | 60 | 0 | 21 | 41 | 0 | 60 |
| Mean | 0 | 20 | 0 | 20 | 20 | 0 | 7 | 13.66 | 0 | 20 |
| % | 0 | 100 | 0 | 100 | 100 | 0 | 31.33 | 68.3 | 0 | 100 |

*EPR: total petroleum ether extract of RCM, ERA: total ethanol extract of RCM, ETH: Ethanol, EAR: total aqueous extract of RCM, FTE: petroleum ether, R1,2,3: Repetitions, V: Alive, M: Death*

**Table 3. Contact toxicity of RCM total petroleum ether extracts**

| Treatment | Repetition | | | Total treatment | Mean treatment | Total (%) |
|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | | | |
| $T_0$ | 0 | 0 | 0 | 0 | 0 | 0 |
| $T_1$ (1%) | 20 | 20 | 20 | 60 | 20 | 100 |
| $T_2$ (0.1%) | 20 | 20 | 20 | 60 | 20 | 100 |
| $T_3$ (0.05%) | 20 | 20 | 20 | 60 | 20 | 100 |
| Total blocs | 60 | 60 | 60 | 180 | 60 | |
| Mean | 15 | 15 | 15 | 45 | 15 | |

**Table 4. Evolution of the number among adults NTG exposed to the treatments**

| Treatment repetition time | $T_1$ (1%) | | | $T_2$ (0.1%) | | | $T_3$ (0.05%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 5 | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 7 | 7 | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 7 | 8 | 7 | 2 | 1 | 2 | 0 | 0 | 0 |
| 6 | 8 | 8 | 9 | 5 | 5 | 4 | 0 | 0 | 0 |
| 7 | 12 | 11 | 10 | 6 | 7 | 5 | 0 | 0 | 0 |
| 8 | 15 | 14 | 15 | 8 | 7 | 7 | 0 | 0 | 0 |
| 9 | **20** | 18 | 19 | 12 | 11 | 10 | 1 | 2 | 2 |
| 10 | 20 | **20** | **20** | 15 | 13 | 14 | 3 | 2 | 3 |
| 11 | 20 | 20 | 20 | 15 | 15 | 16 | 3 | 4 | 4 |
| 12 | 20 | 20 | 20 | 16 | 17 | 16 | 5 | 5 | 4 |
| 13 | 20 | 20 | 20 | 18 | 17 | 17 | 8 | 7 | 7 |
| 14 | 20 | 20 | 20 | **20** | **20** | 19 | 11 | 9 | 10 |
| 15 | 20 | 20 | 20 | 20 | 20 | **20** | 12 | 13 | 14 |
| 16 | 20 | 20 | 20 | 20 | 20 | 20 | 17 | 15 | 16 |
| 17 | 20 | 20 | 20 | 20 | 20 | 20 | 17 | 16 | 17 |
| 18 | 20 | 20 | 20 | 20 | 20 | 20 | 19 | 18 | 19 |
| 19 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 19 | **20** |
| 20 | 20 | 20 | 20 | 20 | 20 | 20 | **20** | **20** | 20 |

**Table 5. Avoidance test of the treatments**

| Repetition | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | $T_1$ (1%) | | $T_2$ (0.1%) | | $T_3$ (0.05%) | |
| | Surface treated | Surface non-treated | Surface treated | Surface non treated | Surface treated | Surface non treated |
| $R_1$ | 5 | 15 | 9 | 11 | 10 | 10 |
| $R_2$ | 0 | 20 | 7 | 13 | 9 | 11 |
| $R_3$ | 5 | 15 | 7 | 13 | 7 | 13 |
| Total | 10 | 50 | 23 | 37 | 26 | 34 |
| % | 66,7 | | 38,33 | | 13,33 | |

According to the table, after two hours of exposure the different treatments of RCM total petroleum ether extracts have caused respectively 66.7, 38.33 and 13.33% repulsion of NTG.

The Table 6 shows clearly that the petroleum ether and Ethyl acetate solvent system (5:1) has revealed 10 spots with Rf ranging from 0.1 to 0.975. While the solvent system (9 :1) shows eight spots revealed with a Rf ranging from 0.2 to 0.975. The first solvent system (5:1) therefore seems to be the most suitable because it has the best separation of compounds.

## 4. DISCUSSION

The results of the extraction from 100 g powder dry leaves of RCM with 600 mL of each solvent, allowed us to obtain an extraction rate of 2.8% for the total ether

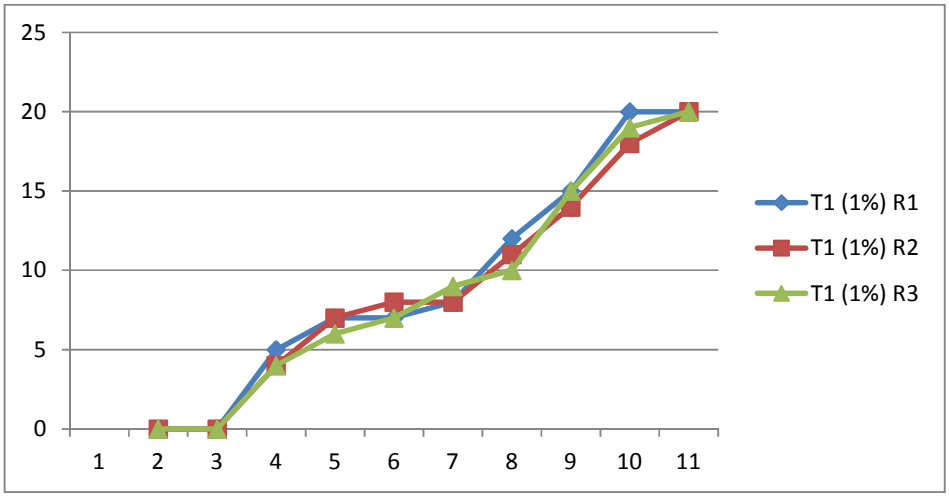


**Fig. 4. Evolution of the number of adults NTG exposed to 1 % RCM total petroleum ether extracts**

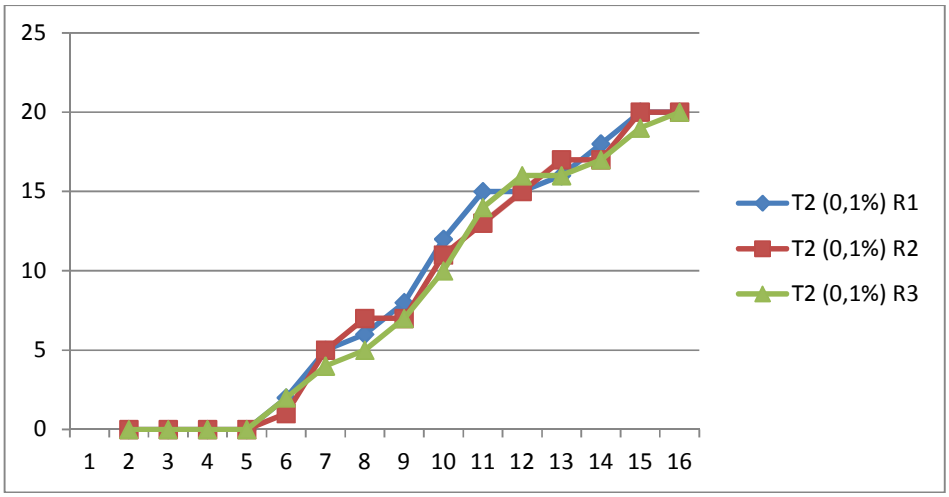


**Fig. 5. Evolution of the number of adults NTG exposed to 0.1 % RCM total petroleum ether extracts**

extracts, 3.6% for the total aqueous extracts, and 8.6% for total ethanol extracts. The high rate of the ethanol extract could be due to an abundance of chemicals soluble in ethanol [14].

The preliminary toxicity test on insects from various extracts showed strong insecticidal activity of total petroleum ether extracts, which resulted in 100% mortality against 31.33% for the ethanol extract and 0% for the aqueous extracts. This result gave an indication that the active molecules are in large quantity in total petroleum ether extracts.

The contact toxicity of the active extracts caused 100% of death in the insect population. The same observation was made for all the treatments: T1 (1%), T2 (0.1%), and T3 (0. 05%). The peak of mortality is reached between 9 and 10 hours for T1, 15 and 16 hours to T2, and 19 and 20 hours to T3. The variation of hours may be due to the concentration of the active molecules on the filter paper.

The avoidance test showed a repellent effect with an average rate of 39.5% of repulsion. This rate puts the RCM in Class II (PR = 20.1 to 40%), as ranked by McDonald et al. [17]. RCM has low insect activity on the NTG. This could be due to the fact that the active molecules in RCM are not much volatile.

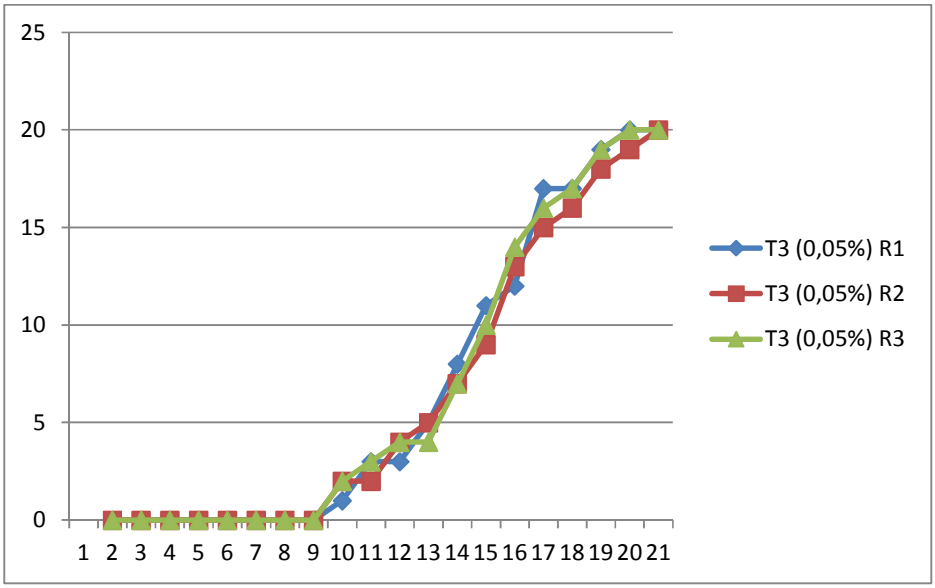


**Fig. 6. Evolution of the number of adults NTG exposed to 0.05% RCM total petroleum ether extracts**

**Table 6. TLC of RCM *in* ethyl acetate-petroleum ether solvent system**

| Petroleum ether solvent and Ethyl acetate system (5:1) | | | Petroleum ether solvent and Ethyl acetate system (9:1) | | |
|---|---|---|---|---|---|
| **Number of spot** | **Distance of the spot (cm)** | **Rf** | **Number of spot** | **Distance of the spot (cm)** | **Rf** |
| 1 | 0.4 | 0.1 | 1 | 0.8 | 0.2 |
| 2 | 0.8 | 0.2 | 2 | 1.2 | 03 |
| 3 | 1.3 | 0.325 | 3 | 1.6 | 0.4 |
| 4 | 1.6 | 0.4 | 4 | 1.8 | 0.45 |
| 5 | 1.8 | 0.45 | 5 | 2.1 | 0.525 |
| 6 | 2.4 | 0.6 | 6 | 2.5 | 0.625 |
| 7 | 2.8 | 0.7 | 7 | 2.8 | 0.7 |
| 8 | 3.2 | 0.8 | 8 | 3.9 | 0.975 |
| 9 | 3.5 | 0.875 | - | - | - |
| 10 | 3.9 | 0.975 | - | - | - |

Analysis of the active fraction, by thin layer chromatography, identified the presence of ten spots for the petroleum ether and acetate ethyl solvent system (5:1) and 8 spots for the solvent system (9:1). According Gareth [18] non-polar solvents such as hexane and ether oil has the ability to extract the following molecules: Fats, essential oils and volatile. Therefore, it makes sense to assign different spots observed to these groups of secondary metabolites.

## 5. CONCLUSION

The results showed that the castor leaves have high insecticidal activity, 100% mortality in 20 hours on NTG, and low insect activity, an average rate of 39.5% in 2 hours.

We recommend supplementary studies in order to determine the lethal concentration (LC50) of the active fraction in RCM.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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