Evaluation of antifungal activity of Moringa oleifera and Jatropha curcas extracts as a natural fungicide against adults of Acanthoscelides Obtectus

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***Abstract*— In the optics to fight against the weevil Acanthoscelides obtectus, the devastating main thing of the bean (Phaseolus vulgaris L.) stored in Kivu and to reduce the exhibition of the organisms not targeted to dangerous insecticides, alternative methods are required. At this point of view, studie on the effect of Saponins and Steroids extracted from the sheets of Moringa oleifera and from Jatropha curcas was led to compare the efficiency of the extracts of these two plants in comparison with an organophosphate commercial insecticide (Malathion) used as witness positive on weevil. The extracts Saponines of, the Moringa caused a maximal mortality of the order of 86,1±1,1 % to a dose 1,5 g/ml followed Steroids extracts of Jatropha with a 69,0±1,7 % mortality rate, whereas the Malathion showed itself more successful by eliminating insects at 92,9±1,9 % and at a dose of 0,4 g/ml after 72 hours of exhibition.**

***Index Terms*— Bean, Acanthoscelides obtectus, Sapinins, steroids, malathion and insecticide plants**

I. INTRODUCTION

The weevil of bean (*Acanthoscelides obtectus*) is one of the most important species on stocks of cowpeas (Phaseolus vulgaris L.) [1,2]. She is met in tropical and subtropical zones. She is present in Kivu where she causes not insignificant damages as well in field as in the stores of storage. During the storage, this devastating beetle drills grains beads and affects their market value. It is the devastating main thing which attacks the bean in fields and which pursues its action in the places of storing [3]. The storage lasts three at five months, a period during which the stored bean its often attacked by it devastating [4].

According to Bouchikhi Tani 2010 [5], losses in weights caused in the stocks can be estimated at more than 80 % after six or seven months of storage and cannot be anymore consumed. These insects perforate the kept seeds and so decrease their commercial values.

In tropical Africa, the losses caused by these insects are estimated between 30-80 % of the fact that the post-embryonic development is made in seeds, causing on important damages in the food stocks as well as during the storage of seeds. She can have until six generations a year in the tropical regions; while in the seed of the bean, 1 at 28 larvae develop and consuming completely the contents of the seed [2].

To controlate devastating effect without the use of pesticides of synthesis, he is interesting to find alternative methods in the phytosanitary protection. Indeed, new products are looked for for, on one hand, to assure an effective protection of the agricultural production, and on the other hand, to contribute to a sustainable management of the environment from this perspective, the use of extracts of plants endowed with insecticidal activities offers a certain potentiality [6]. Numerous works highlighted the negative effects of the extracts of plants on beetle. So, for example, extracts of *Melia azaderach* L., *Azadirachta indica* A. *Juss* affects the fertility and the mortality of *tabaci B.* [7-9]; soya oils and grains of the cotton plant also showed themselves toxic to the *white fly* [10]

It's in this context that we tested the insecticidal activity of saponins and steroids extracted from *Moringa oleifera* and *Jatropha curcas* against the *Acanthoscelides Obtectus* pest of the cowpea. These are generally selective, non-hazardous insecticides for the man and environment, biodegradable and less expensive than insecticides of synthesis [11-14].

II. MATERIALS AND METHODS

A. Plant material

We used on one hand the local variety of bean (*Phaseolus vulgarus*) for the breeding of our insects and on the other hand, the insecticidal plant leaves of *Moringa oleifera* and *Jatropha curcas*. These leaves were harvested in the province of South Kivu in the plain of the Ruzizi in the east of the Democratic Republic of Congo during the month of may 2016. The sheets were cut, weighed and spread in thin layers over the ground for the drying at room temperature during a week. Sheets are crushed into powder by means of an electric crusher and the obtained powder is weighed to determine the increasing doses to be prepared.

B. Animal material

It's composed by insect pest of *Acanthoscelides Obtectus*. The breeding is realized in plastic jars in which were added a sufficient number of insects, indefinite sex. These plastic jars have been kept at the ambient temperature of the laboratory approximately 26 ± 1°C. The infested grains are left in incubation until the appearance of the new adult insects. The adult insects used for the tests are obtained from these breedings of mass. The individuals were collected by sieving of the seeds of bean infected.

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C. Extraction of chemicals compounds from the Moringa oleifera and Jatropha curcas extracts

The extractions are made after a maceration of 24 hours, then the filtration and waste is kept in glass jars. The filtrate is served in the identification of the families of chemical substances.

D. Phytochemical screening of plants extracts

Phytochemical analysis was carried out on the basis of tests for coloring to highlight the major chemical groups. This test focused on maceration extracts of hexane, methanol and aqueous extracts of Moringa and Jatropha plants. Chemical groups were identified according to the methods described by Nemlin and Brunel [15,16]. The results have been classified according to: Highly positive: +++; Fairly positive: ++; weakly positive: +; Negative test: -

***Alkaloids*.** Two drops of Bouchard's reagent (reagent of iodine-iodide) were added to 1 mL of each extract. A red-brown precipitate indicated a positive reaction. *Saponins*. The potassium dichromate (1%) tests acidified with sulfuric acid (98%) and the foam tests carried out on the aqueous extract were used to identify them. On the one hand, the appearance of the dirty green coloring for the first test and the persistent foam following vigorous agitation for the second test testify to the presence of the saponins [17]. ***Glycosides*.** Glucosides were identified from the aqueous extracts by the Fehling reagent test ($CuSO_4.5H_2O$ plus potassium and sodium double tartrate in distilled water) acidified with 1% HCl. The formation of a red-brick precipitate testifies to the presence of glucosides.

Fkavonoid. In a glass tube containing 2 mL of extract, a few drops of 10% NaOH were added. Appearance of yellow-orange color indicated the presence of flavonoids in the sample [18]. ***Therpenes*.** The terpenes were identified on the basis of the Hurschson reagent (acetic acid) test carried out on the ethereal organic extract. The presence of the yellow color turning red indicates the presence of the terpenes. ***Steroids*:** A few drops of concentrated H_2SO_4 were added to 2 mL of extract, the apparition of green mauve color indicated the presence of steroids [17]. ***Tannins*.** The tannins are determined by the Stiasny reagent test (1% ferric chloride) carried out on the aqueous extract. The formation of blue, blue-green, blue-dark or green coloring indicates the presence of tannins [18,19].

***Quinones*.** An aliquot of extract was dissolved in 5 mL of diluted HCl and heated in a boiling water bath 20 minutes, and then extracted with 20 mL of chloroform after cooling. The organic phase was added with 0.5 mL of 50% hydroxide ammonium diluted solution. The red to violet changing color indicate the positivity reaction [19]. ***Phenols*.** Phenols were detected by the $FeCl_3$ (1%) and sulfuric acid (98%) tests conducted on the ethanolic organic extract. The formation of the dark red color indicates the presence of the phenols. ***Cartenoids*.** The carotenoids were detected on the ethereal organic extract by the test with 1% hydrochloric acid and sulfuric acid (98%). The appearance of the green-blue color indicates the presence of the carotenoids [17,18].

***Amino acids*.** The amino acids are identified by the ninhydrin developer which forms a violet product with hot amino acids. On a filter paper, place a drop of the solution. At the same place drop a drop of the reagents with ninhydrin and heat the filter paper in an oven at 90 ° C for 5 minutes. The appearance of a blue or violet shade distinctly different from that of the blank test indicates a positive test [17,18]. ***Coumarins*.** To 5 mL of extract, 2 mL of hot water were added, and then the solution was shared between two test tubes. In the content, we added 0.5 mL of 25% NH_4OH. Under the UV light (366nm), an intense fluorescence indicated the presence of coumarins [17]. ***Anthocyanins*.** Five milliliters of 10% H_2SO_4 was added to 5mL of 5% extract, and then a base (five drops of 25% NH_4OH) was added. If the coloration is accentuated by acidification and then change into blue-purplish in a basic environment, anthocyanins were present [17,19].

E. Extraction of insecticide organic solutions

Residues obtained during the preparation of extracts so organic as aqueous, were of use to the extraction of insecticidal organic substances such as saponins and steroids, according to the classic methods [20-22]. Saponins and steroids were extracted by based on itself on their solubility in the ethanol. After filtration of the organic extracts and the vacuum evaporation of the liquid phase, the obtained black residue was getting back with a mixture chlorobenzène-water (2/1). He appears a dark phase containing the steroids which are then separated by decantation then evaporated dry. The residue of steroids obtained was got back in 2 ml of water distilled for the test of insecticide activity. Saponins is extracted based on itself on their insolubility in the water and their solubility in the ethanol. After filtration of the organic extracts ethanoic and vacuum evaporation of the liquid phase, the black-brown residues so obtained will be streamlined with 3ml of hexane and then to evaporate dry. This recently obtained residue will again be dissolved in the mixture methanol-water (4/1).

Saponins contained in this solution is precipitated by 2ml of diethyl ether, after a 3rd evaporation, a brown-black residue is one more time obtained then to get back by 2 mL some methanol-diethyl ether (2/1). This last obtained solution is spin-dried during 5 minutes in 3000 tours / second then, rest during 24 hours before separating the phase at the bottom with the floating phase containing saponins. Finally, the vacuum evaporation of the latter gives a dry residue of the saponins of colors brown-black who will be got back by 2 ml of distilated water for the toxicity test.

F. Insecticide activity test

After extraction of active principles and weighed the black residues diverse doses of extracts were prepared for the test of insecticidal activity. For *Moringa oleifera* extact, 3 g of black residues are obtained for Saponins and from 3,5 g for steroids. Whereas for *Jatropha curcas*, we obtained 3 g of saponins and 4g of steroids. For Malathion, 20g are diluted in 50ml with distilled water. So the black powder residues obtained were diluted in 2mL of distilled water to constitute the first concentration 10^{-1} of this solution is diluted in 9 mL of water distilled to train the 2nd concentration 10^{-2}.

Several doses (D1, D2 and D3) from extracts of the plant Moringa oleifera were prepared respectively 1,5g/mL; 0,15 g/mL and 0,015 g/mL for saponins and 1,75 g/mL, 0,17g/mL and 0,017g for steroids. For Jatropha Curcas, we have 1,5g/mL ; 0,15 g/mL et 0,015 g/mL for saponins and 2 g/mL, 0,2 g/mL, 0,02 g/mL for steroids. As a witness, we used the Malathion 0,4 g/mL, 0,04g/mL and 0,004g/mL. 1 ml of every solution to given dose was deposited on a quarter of filter

paper by means of a micropipette. After evaporation of the solvent outdoors, the filter paper was introduced into the experimentation box [17].

Five grams of seeds bean are introduced in every box. These seeds were used as substratum for insects. So, 20 individuals of Acanthoscelides Obtectus, were placed in every bottle containing active ingredients. Bottles are closed by means of a muslin finally to avoid the leakage or death of insects by asphyxiation. Boxes were kept in a well aerated place. Time of exposure was fixed at 24, 48 and 72 hours. During the counting, the insects which incapable to move after the duration of the test (24, 48 hours and 72 hours), were considered died.

G. Data analysis

The R software was used for the analyses of the variance.

III. RESULTS

A. Pre-test of insecticide activity

The results of the pre-test of the extracts of our plants are resumed in table 1.

Table 1. Mortality rate after 72 hours exposition of insects to the extracts of Moringa and Jatropha

Extracts	Mortality rate (%)
1. Aquous extract [a et b]	100
2. Organic extact [a et b]	100
3. Water	0

The letters a and b mean: a: *Moringa* and b: *Jatropha*

Compared with the negative telltale (water), all the extracts (moringa and jatropha) presented after 72 hours of contact a very big insecticidal activity on *Acanthoscelides Obtectus*, pest of the cowpeas in stock.

B. Phytochemical Screening of the extracts of sheets of Moringa and Jatropha

The various active principle identified in the extracts of various plants are presented in the table 2.

Table 2. Phytochemical screening of *Moringa* and *Jatropha* extracts

Chemicals compounds	Results	
	Moringa	**jatropha**
Alkaloids	+++	+
Saponins	+++	++
Phenols	+	++
Quinones	+++	+
Tannoids	-	-
Terpenoids	-	-
Glycosides	-	-
Steroids	+++	+++
Flavonoids	-	-
Carotenoids	+	+
Anthocyanins	-	++
Proteins	++	++
Amino Acids	++	++
Coumarins	++	++

Highly positive: +++; Fairly positive: ++; weakly positive: +; Negative test: -

This table presents the results of the qualitative analysis of the extras of *Moringa oleifera* and *Jatropha curcas*. With regard to this table we find that for *Moringa oleifera* the saponins, quinones, steroids, alkaloids and carotenoids are strongly present, followed by the amino acids, proteins and coumarins which are weakly present, phenols weakly present while tannoids, terpenoids, glucosides, flavonoids and anthocyanins are absent. As for *Jathropha curcas*: saponins, steroids and alkaloids are strongly present; phenols, anthocyanins, proteins, amino acids and coumarins are averagely present; tannoids and carotenoids are weakly present whereas quinones, flavonoids, terpenoids, glucosides are absent.

C. Effects of the various extracts doses on insects

Table 3 shows the mortality rate of A. Obtectus according to the duration of application of insecticides, according to the extracts of the plants.

When the insects are brought into contact with the extracts, we observe after 24 hours of exposure the first effects of agony and death of the insects.

Table 3: Mortality rate of weevil during exposure

Exposure time	Dose								
	D1			D2			D3		
	Saponins	Steroids	Malathion	Saponins	Steroids	Malathion	Saponins	Steroids	Malathion
24h	71,1±0,4	64,4±0,3	90,1±1,1	53,3±0,2	33,3±0,3	75,6±1,2	24,4±0,2	15,6±0,1	57,8±0,5
48h	82,0±0,8	68,0±0,1	93,7±1,4	59,2±0,1	34,3±0,2	79,3±0,8	25,2±0,1	16,0±0,0	59,1±0,7
72h	86,1±1,1	69,0±1,7	92,9±1,9	60,0±0,0	38,7±0,4	81,1±0,2	28,1±0,1	17,0±0,1	60,0±0,2

It ensues from this table that insect mortality decreases with increasing dilutions and increases with the duration of exposure of the weevil according to the different doses applied. Further to it, positive control Malathion showed itself more successful by eliminating insects to an average of 92.9 ± 1.9, followed by Saponins extracted from *Moringa oleifera* (86.1 ± 1.1) the steroids extracted from the *Jatropha Curcas* come in third with an average of (69.0 ± 1.7). It is the dose D1 which turned out the most effective that doses D2 and D3 compared with the pesticide of synthesis which is the Malathion. The efficacy of our extracts as insecticide appeared after 72 hours of exhibition. Of the point of seing efficiency between Moringa and Jatropha, the Moringa extract has an insecticidal effect more pronounced than the extract of Jatropha.

Table 4. Lethal doses (g/ml) of saponin and steroid residues extracted from *Moringa* and *Jatropha* against weevil

Vegetable	Extracts	DL50	DL90
Moringa	Saponins	3,34±0,01	4,44±0,03
	Steroids	2,33±0,01	3,67±0,02
Jatropha	Saponins	0,23±0,01	0,53±0,01
	Steroids	1,54±0,03	3,34±0,01

The table above shows that LD50 lethal doses vary from one insecticidal organic substance to another. However, steroids have a low LD50 lethal dose compared to saponins for Moringa. For our results, saponin is best adapted for the conservation of beans.

IV. DISCUSSION

The results of this study attest to the presence of substances looked for in both plants, table 2 shows a strong presence of Steroids and Saponins and an average presence of alkaloids, lipids, protides, amino acids of quinones and coumarins but also of carotenoids, phenols and anthocyanins. This presence confirms the therapeutic virtue of the extracts of Jatropha curcas but also the efficacy of the extracts of different parts of this plant, as well as its oil in the fight against the pests of weevils [23,24]. The results of the various experiments showed a significant mortality of weevil by the products used. Insect mortality is a function of the dose of the extract and the time of exposure to the product. The efficacy of the products was maximal after 72 hours of exposure. Both plants (*Jatropha curcas* and *Moringa* oleifera) contain substances with insecticidal effect. What joins Bambara 2008 [6]; having confirmed the insecticidal virtues of plants and showed the presence of several insecticidal substances in many plants in the natural state.

V. CONCLUSION

The concentrations used in this study for plant extracts (Moringa and Jatropha) were compared to a synthetic insecticide which is Malathion in order to test the efficacy of our products. Results show that the saponins extracted from Moringa are more active than the steroids extract of Jatropha. In the short term, the synergistic effect of saponins and steroids can be used to effectively control of weevils (*Acanthoscelides obtectus*). These results allow to recommend the use of the extracts of *Jatropha* and *Moringa* because their efficiency is demonstrated. Thus, in view of the low cost of production of these extracts, its non-toxicity to humans (animals) and the environment; we suggest use instead of synthetic insecticides (Malathion) that cause a lot of damage in the environment.

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