Evaluation de l’effet insecticide des formulations a base des graines de *Moringa Oleifera* sur *Acanthoscelides Obtectus*

[ Insecticidal activity of formulations based on *Moringa Oleifera* seeds on *Acanthoscelides Obtectus* (Coléoptera: Bruchidae) ]


¹University of Dschang, Cameroon  
²University of Yaounde I, Cameroon  
³University of Douala, Cameroon  
⁴Institut of Agricultural Research for Development, Cameroon  
⁵University of Yaounde II, Cameroon

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**ABSTRACT:** Stored seeds of *Phaseolus vulgaris* L. in are the target of several insects like *Acanthoscelides obtectus* Say. These pests can cause losses greater than 80 % after six to seven months of storage. The present study aimed to evaluate the insecticidal effects of *Moringa oleifera* seeds on adults of *A. obtectus* in storage through two compositions (ie powdery and oily). Four concentrations of oils (3.33; 6.66; 9.99 and 13.33 μL/g), four powder concentrations (4; 16.67; 33.33 and 50 %) and one negative control (C₀ = 0 μL / mL) for each composition were used. The experiment was conducted in the laboratory, with four repetitions. The results of the oil contact toxicity test revealed 100 % mortality at doses of 300 and 400 μL after two days of exposure. As for *Moringa* powder, the insecticidal effect was less pronounced for all doses with a mortality rate of 55 % at day 5 for doses 10 and 15 g. The inhibition of eggs laid and their viability is a function of the concentration of *Moringa* oil. On the other hand, the powder had no effect on the number of eggs laid and rather stimulated the viability of the eggs. *Moringa* oil prevents weight loss of stored bean seeds with a null loss percentage at doses of 300 and 400 μL. As for the powder, it had no effect on the weight loss of bean seeds in storage. The powder and oil of *Moringa* seeds did not affect the germination capacity of the seeds for all the doses tested. *Moringa* seed oil can therefore be exploited in the integrated control of the pest of common bean seeds in storage.

**KEYWORDS:** *Acanthoscelides obtectus*, *Moringa oleifera*, *Phaseolus vulgaris*, oils, powder, insecticidal effect.

1 **INTRODUCTION**

Developing countries account for almost 90 % of human consumption of pulses mainly in the form of dry legumes. Indeed, legume seeds contain two to three times more protein than cereals and including all amino acids (except sulfur amino acids) essential for human nutrition, which can offset the lack of animal protein [1]. Among legumes, the common bean (*Phaseolus vulgaris* L.) is prominently represented, whose dry seeds, cultivated and consumed all over the world, constitute a major source of protein [2]. *P. vulgaris* plays an important role in the household economy and in the food security of populations [3]; moreover, production costs are lower than those of cereals.

**Corresponding Author:** Kone Nsangou Abdou Nourou
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The bean crop is the second most cultivated legume after groundnuts in Cameroon and occupies about 296,371 hectares with an estimated production of 366,463 tons per year [4]. Beans are consumed in Cameroon-major cities and exported to neighboring countries such as Gabon and Central African Republic.

However, in addition to yield losses in the field, beans suffer considerable losses during storage, due to the lack of control over storage conditions. The main pests of stocks are weevils. Of these, Acanthoscelides obectus is proven to be the weevil that causes the highest damage to common bean seeds in storage. During its development, it deteriorates the nutritional value of bean grains, affects its germination capacity, its organoleptic character, and consequently its economic value.

To cope with these problems and to remedy post-harvest losses, many producers resort to the use of synthetic pesticides, despite the risks of toxicity posed by some of them [5], particularly on humans and the environment. In addition, some pests have developed significant resistance to these products [6].

Due to the consequences of chemical pesticides, the development of safe alternative control strategies that are affordable and respectful of humans and the environment becomes necessary. Many studies have shown the effectiveness of plants in the conservation of stored seeds against insects. This is the case of leaves of Thymus vulgaris L. on A. obectus [7], leaves of Moringa oleifera Lam, seeds of Senna tora and seeds of Piper nigrum on C. maculatus [8], [9], [10] the roots of Mondia whitei on A. obectus [11]. It is in this context that the present study was conceived with the main aim to evaluate the effect of the oil and powder of moringa seeds on bean weevils in beans under storage conditions.

2 MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 STUDY SITE

The present study was conducted in the Plant Biotechnology Laboratory, Phytopathology and Plant Protection Research Unit of the Department of Plant Biology, Faculty of Sciences, University of Yaoundé I.

2.1.2 PLANT MATERIAL

Three varieties of beans were used in this study: white kidney, small black dwarf grain, and LPG 190 C. For breeding weevils Moringa seeds were used for different toxicity tests.

2.1.3 ANIMAL MATERIAL

The experiment is carried out on the adults of Acanthoscelides obectus, this choice of animal material is justified by the importance of the damage of this pest which infests seeds of P. vulgaris stored. A. obectus is maintained by mass rearing on bean seeds in ambient conditions.

2.2 METHODS

2.2.1 REARING OF WEEVILS

Mass rearing of Acanthoscelides obectus was done with A. obectus individuals collected from infested seeds at a local provision store. About 500 adult weevils were introduced into a bag containing healthy bean seeds. The bag was closed with elastic fasteners and kept in ambient conditions for twenty days (time required for perfect oviposition). Subsequently, the culture medium was sieved through a sieve with a mesh size of 4 mm in order to rid it of all living and dead insects and kept under the same conditions for ten days to allow for the emergence of a new generation of adult insects.

2.2.2 OBTENTION OF MORINGA POWDER AND OIL

To obtain moringa powder, dry moringa seeds were rid of their shells and crushed using a manual mill shortly before the various tests. For the oily composition, 368 g of moringa seed powder was weighed and loaded into cartridges and mounted on soxhlet. One and a half liter (1.5 L) of acetone was added for 72 hours. The product obtained was then mounted on a rotary evaporator under pressure at the solvent evaporation temperature (acetone) in order to eliminate the solvent. The oil obtained
was weigh and put in a bottle and stored in the refrigerator for the various tests. The extraction yield was calculated using the formula of [12].

\[ \text{Yield} \% = \frac{\text{mass of oil (g)}}{\text{mass of plant material (g)}} \times 100 \]

### 2.2.3 Repulsion Test Of Moringa Oleifera Seeds Oil On A. obtectus

The repulsion test was used to calculate the percentage repulsion of *M. oleifera* seed oil on bean weevil with the preferential zone method described by [13]. In fact, the 9 cm diameter filter paper discs (Whatman number 1) were divided into two equal parts of 31.8 cm² of surface. Four concentrations were prepared according to a geometric progression of reason of 2 by dilution in 0.5 ml of acetone of the following volumes of Moringa seed oil: 100, 200, 300, and 400 μl. The solutions obtained were homogenized by manual stirring and uniformly spread over one half of the disc using an insulin syringe. The other half, treated only with 0.5 ml of acetone, served as a control. Ten minutes later, the filter paper discs were reconstituted by connecting the treated halves and the control halves with clear adhesive tape and placed in petri dishes. Fifteen adult weevils, three days old, were placed at the center of each filter paper and the petri dishes were covered. Four repetitions were performed for each of the oil concentrations. Counting of the weevils on each half disk was performed after 2 and 4 hours of treatment under laboratory conditions. The repulsion percentage was then calculated according to the formula used by [14] as follows:

\[ \text{RP} \% = \left(\frac{\text{No. of hairs present on the half-disk treated with acetone} - \text{No. of weevils present on the half-disk treated with the oil dose}}{\text{No. of hairs present on the half-disk treated with acetone} + \text{No. of weevils present on the half-disk treated with the oil dose}}\right) \times 100 \]

RP: repulsion percentage  
Nac: number of hairs present on the half-disk treated with acetone  
N0: number of weevils present on the half-disk treated with the oil dose

The average repulsion percentage was calculated and assigned to the different repulsion classes according to the classification of [15] represented by the following table:

<table>
<thead>
<tr>
<th>Repulsion class</th>
<th>Repulsion interval (%)</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 0</td>
<td>PR &lt; 0.1</td>
<td>Not repulsive</td>
</tr>
<tr>
<td>Class I</td>
<td>0.1 – 20</td>
<td>Very weakly repulsive</td>
</tr>
<tr>
<td>Class II</td>
<td>20.1 – 40</td>
<td>Weakly repulsive</td>
</tr>
<tr>
<td>Class III</td>
<td>40.1 – 60</td>
<td>Moderately repulsive</td>
</tr>
<tr>
<td>Class IV</td>
<td>60.1 – 80</td>
<td>Repulsive</td>
</tr>
<tr>
<td>Class V</td>
<td>80.1 – 100</td>
<td>Highly repulsive</td>
</tr>
</tbody>
</table>

### 2.2.4 Inhalation Toxicity Test Of Moringa Oleifera Seeds Oil On A. obtectus

The inhalation effect of *M. oleifera* seed oil was investigated in adults of *A. obtectus*, adopting the method described [15]. In glass jars (500 ml capacity) cotton masses were fixed with a thread at the center of the watertight lids. Doses of *Moringa* oil: 100, 200, 300 and 400 μl, corresponding to the concentrations calculated for 200, 400, 600 and 800 μl/L of volume of air, were injected in the cotton mass. At the same time, a control that did not receive oil (0 μl/L of air volume) was put in place. Fifteen adult weevils aged five days or less were quickly put inside the jars and immediately closed hermetically. Four repetitions were performed for each dose and for the control. The count of dead insects in each jar was done after 24 h, 48 h, 72 h, and 96 h while always keeping the jars close.

### 2.2.5 Contact Toxicity Test On Phaseolus vulgaris Seeds Oil And Moringa Powder On Acanthoscelides obtectus

To 30 g of common bean seeds (white kidney cultivar) contained in transparent polystyrene jars were added different doses of oil (100, 200, 300 and 400 μl) and powder (1, 5, 10 and 15 g) of *M. oleifera* seeds, and the whole mixture was shaken to assure proper mixing. The respective concentrations of 3.33, 6.66, 9.99 and 13.33 μl/g for the oil and 4, 16.67, 33.33 and 50 %
of the seed weight for the powder were obtained according to a geometric progression of reason of 2. Then, 15 adult insects were introduced into each jar and the latter was covered with porous tissues held with elastic fasteners. The control boxes, prepared under the same conditions, were treated only with acetone (1 ml) in the case of the oil and untreated in the case of the powder. Four repetitions were carried out for the tests and for the control (powder and oil) and the jars were kept in the laboratory.

Dead insects were counted every 24 hours for a period of 5 days. The mortality rate was evaluated using the following formula:

\[ MR (\%) = \left( \frac{NDW}{NIW} \right) \times 100 \]

Where:  
MR (%): Mortality rate; 
NDW: Number of dead weevils; 
NIW: Number of introduced weevils.

All eggs laid on the seeds and in each jar were recorded using a binocular louse after 14 days of treatment. Adults which began to emerge from day 28 to day 45 were counted regularly and removed from the jars. The seed viability rate was calculated according to the formula of [7].

\[ Viability \ Rate = \frac{\text{number of emerged adults}}{\text{number of eggs laid}} \times 100 \]

At the end of the emergence of adults of the first generation, the loss in weight of grains after undergoing the different treatments (powder and moringa oil) was evaluated according to the formula used by [7].

\[ Weight \ loss (\%) = \left\{ \frac{\text{initial weight}}{\text{final weight}} \right\} \times 100 \]

At the end of the experiment, 50 seeds of each batch tested (powder and oil), taken at random, were nursed in petri dishes containing cotton soaked in water. A control batch for each composition (oil and powder) was tested, two to three days after the experiment, the germinated seeds were counted and the germination rate was calculated using the following formula:

\[Germination \ rate = \frac{\text{number of seeds germinated}}{\text{total number of seeds}} \times 100\]

3 DATA ANALYSIS

Data obtained were submitted to Fisher-Snedecor (ANOVA) test at p = 5 % one-factor threshold using the X-LStat (version 6.0 software). An additional test of Newmann and keuls at the 5 % threshold was carried out in order to determine the homogeneous groups. Graphs and tables were drawn using the Microsoft Excel Spreadsheet 2016.

4 RESULTS

4.1 YIELD OF OIL EXTRACT

The methods described above made it possible to obtain a yellowish powder with a relatively homogeneous and fine grain size and 1.5 ml of yellow oil for an extraction yield of 26.25 %.

4.2 MORINGA OLEIFERA SEED OIL REPULSION TEST ON A. OBTECTUS

The filter paper repulsion test of moringa oil on A. obtectus adults after two and four hours of exposure showed that M. oleifera seed oil had no repellent effect on A. obtectus. This oil belongs to the repulsion class 0 according to the classification of [13] because its repulsion interval is less than 0.1 %.

4.3 INHALATION TOXICITY TEST OF MORINGA OLEIFERA SEEDS OIL ON A. OBTECTUS

By inhalation of moringa oil vapors, no deaths were recorded on all batches tested and for all doses after 96 hours of exposure. Thus, Moringa oleifera seed oil was not active by inhalation (fumigation) on the mortality of A. obtectus adults for the tested concentrations.
4.4 Contact Toxicity Test on Phaseolus Vulgaris Seeds of Oil and Moringa Powder on Acanthoscelides Obectus

The percentage of crude mortality of *A. obtectus* adults subjected to different doses of *M. oleifera* oil by seed contact varies with the days and the dose applied. There was a significant difference between the different doses of oil tested and the control according to the Newmann and Keul test at 5 % threshold. A 100 % mortality rate at the second day of exposure was observed for doses 3 and 4 (which doses), whereas the control only recorded a mortality rate of 41.667 % after five days of exposure. In the case of batches treated with moringa powder, the mortality rate increased with an increase in the different doses and the exposure time. There was a significant difference between the control and the various powder treatments at the threshold of 5 %. On day 5, the mortality rates were 75 % for dose 1 and 2, 80 % for doses 3 and 4, and 55 % for the control, respectively (fig 5).

4.5 Number of Eggs Laid

The number of eggs decreased with increase in the dose of moringa oil. No eggs were observed for doses 3 and 4. Doses 3 and 4 recorded 57.75 ± 15.49 and 21.75 ± 15.49 eggs laid, respectively and the control, 229.75 ± 29.35. The powder of moringa seeds affected the oviposition of *A. obtectus*. Egg numbers of 118 ± 22.23, 118 ± 22.23, 94.25 ± 22.23, 88.75 ± 22.23 and 155.5 ± 22.23 were observed for doses 1, 2, 3, 4 of powder and the control.

4.6 Egg Viability Rate

The viability rate of the eggs laid decreased significantly with increase in the dose of oil. No emergence was observed for doses 3 and 4. The viability rate of dose 2 (19.88 %) was significantly different from that of dose 1 (24.80 %) and both were significantly different from the control (43.60 %) at the threshold of 5 %. The egg viability rate (emergence) was stimulated by...
the presence of moringa seed powder. There was significant difference between all powder doses (67.30; 67.38; 69.15 and 69.65 %, respectively) and the control (50.71 %).

Fig. 3. Average rate of viability of eggs (emergence) of A. obtectus on P. vulgaris treated by contact with different oil doses (A) (T1 = 0 μl; HD1 = 100 μl; HD2 = 200 μl; HD3 = 300 μl; HD4 = 400 μl); and powder (B) (Dose 0: control, Dose 1: 1 g, Dose 2: 5 g, Dose 3: 10 g Dose 4: 15 g) des graines de M. oleifera. The values followed by the same letters do not differ significantly between them at the threshold of 5% (Newman and Keuls test)

4.7 WEIGHT LOSS

Weight loss of the treated seeds decreased with increasing amounts of moringa seed oil used (Fig. 8). Thus, no weight loss was observed for doses 3 and 4; doses 1 and 2 recorded a weight loss of 1.16 % each corresponding to a weight loss of 4.91 % for the control. In the case of powder, weight loss varied very little according to the doses. Weight of 3.67; 3.41; 3.41 and 4.58, respectively, were noted for doses 1, 2, 3 and 4 of the powder and the control.

Fig. 4. Percentage weight loss (%) at different oil doses (A) (T1 = 0μl; HD1 = 100μl; HD2 = 200μl; HD3 = 300μl; HD4 = 400μl) and powder (B) (Dose 0: control, Dose 1: 1 g, Dose 2: 5 g, Dose 3: 10g Dose 4: 15g) seeds of M. oleifera. There is no significant difference between values with the same letters at 5 % threshold (Newman and Keuls test)

4.8 SEED GERMINATION POWER

All tested seeds germinated regardless of the treatment or dose applied. There was no significant difference between the doses compared to the control and those for all treatments (moringa seed oil and powder moringa).

Table 2. Germination rate of treated and untreated bean seeds (control) at different doses of M. oleifera oil and powders

<table>
<thead>
<tr>
<th>Duration</th>
<th>Moringa oleifera oil and powders</th>
<th>Doses of powders</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 μl</td>
<td>100μl</td>
</tr>
<tr>
<td>Day 2</td>
<td>86</td>
<td>82</td>
</tr>
<tr>
<td>Day 3</td>
<td>94a</td>
<td>90a</td>
</tr>
</tbody>
</table>

The values followed by the same letters do not differ significantly at the threshold of 5% (Newman and Keuls test).
5 Discussion

Extraction of 380 g of moringa seed powder with acetone produced a yield of 26.25%. This yield could be attributed to the condition of the plant material at the time of collection, atmospheric conditions [16], to the polarity of the solvent, and to the volume used. This result is contrary to that of [17] who obtained an extraction rate of 40.72% of moringa seed oil with acetone by extraction using Soxhlet.

The inhalation toxicity test revealed that moringa oil is ineffective by inhalation against A. obtectus adults. This could be explained by the absence in moringa oil of volatile secondary metabolites or aromatic compounds or their presence in insufficient proportions to saturate the enclosure. Similar results were obtained by [11].

According to the results of the repulsion test, the oil of moringa seeds belongs to the class 0 opposite adults of A. obtectus, according to Mc Donald’s classification [13]. It contains little or no repulsive compounds such as terpenoids, phenolic compounds, and alkaloids. The same observation was made by [14] for the oils of Dischrotachys glomerata, Echinops giganteus and Mondia whitei against bean weevil.

Both formulations of moringa were toxic to A. obtectus, moringa oil having a faster action than the powder. This could be justified by the fact that certain bioactive compounds acting in the oily formulation are inhibited or have their bio-activities reduced in the powdery formulation by other compounds present in it. Similar results were obtained by [11, 18] respectively on A. obtectus with the essential oils of E. giganteus and M. whitei, and on Sitophilus Zea mays with the powders of Moringa oleifera, Melia azedarach, Annona muricata and Cyrtasperma senegalesis. The decrease in the number of eggs laid according to the moringa oil doses could be attributed to the toxicity of the oil because the increasing mortality of the insects according to the doses did not leave them enough time for oviposition as opposed to moringa powder, which showed no significant difference with the control in the number of eggs laid. Similar results were obtained by [19] on C. maculatus after 72 hours with Moringa, Fenugreek, Jojoba and sweet almond oils.

The emergence of F1’s individuals from A. obtectus decreased with increase in the dose of oil applied, and absent at a dose of 300 μl. Similar results were obtained by [19, 11]. In contrast to oil, Moringa powder instead stimulated the emergence of bean weevil F1. The treated lots showed a viability rate higher than that of the control and those at the lowest dose. This could be justified by the similarity existing between the constituents of bean seeds and that of Moringa, in particular proteins, vitamins and amino acids. These results are similar to those of [9] on C. maculatus with moringa leaf powders. Of the two formulations based on moringa seeds tested, only the oil proved effective in preserving the weight of bean seeds with a loss of 0% observed at a dose of 300 μl. The loss of weight of P. vulgaris seeds observed in the batches treated with the different doses of moringa powder, is related on one hand to the biological activity of the larvae inside their cotyledons and on the other hand to the emergence of adults from the development of these larvae [20]. Similar results were obtained by [11, 7].

At the end of the germination test, all the seeds tested germinated on the second day of the experiment; no significant difference was observed between the doses of each formulation tested and the control. This result could be justified by the volume of seeds of the bean cultivar used for the tests and the period of infestation. In fact, the larvae could consume the food reserves of the cotyledons without affecting the entire germ. These results are contrary to those of [7] with the seeds of P. vulgaris (Kidney white cultivar) treated at different doses of essential oils of lemon, mandarin, bergamot, laurel, eucalyptus, cedar, lavender, peppermint and thyme.

6 Conclusion

Having reached the end of this study which had as main aim to evaluate the insecticidal effect of oil and powder of Moringa seeds on bean seeds in storage. It appears that the two formulations based on the seeds of Moringa oleifera tested are toxic to adults of Acanthoscelides obtectus. However, predominance in effectiveness of the oily formulation was observed from the concentration of 300 μl/30 g of bean seeds with a 100% mortality rate after two days of exposures unlike the powder. Moringa seed oil reduced seed oviposition and the emergence of adult F1 individuals of A. obtectus, unlike moringa seed powder, which had almost no effect on oviposition of seeds, stimulated the emergence of F1 individuals of the bean weevil. Regarding the agronomic characteristics of common bean seeds, our results showed that of the two formulations based on moringa seeds tested (oil and powder), only moringa oil allows the prevention of bean seed weight loss in storage under high doses (300 and 400 μl) with 0% weight loss of bean seeds, unlike moringa seed powder where weight losses of 3.41% and 3% were observed at high doses (10 g and 15 g).
REFERENCES


