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# Chemical characterization and insecticidal effect of *Moringa oleifera* L. seed extract on common bean weevil (*Acanthoscelides obtectus* Say)

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**ABSTRACT:** Some insects cause high losses of the common bean during storage, namely, *Acanthoscelides obtectus* Say. Chemical insecticides are commonly used to control insects, but their overuse adversely affects the environment and consumers' health. There is, therefore, the need for an alternative control method. This study was aimed to evaluate the chemical characterization and insecticidal property of *Moringa oleifera* seed extract on the developmental stages of *A. obtectus* in stored beans. Three types of *M. oleifera* seed extracts, namely methanol, ethanol and acetone extracts, were used at doses of 12.5, 25 and 50 µl/ml each. HPLC-MS was used to characterize these extracts. The toxicity of extracts against the adults, the number of eggs and the viability rate of laid or emerged eggs of *A. obtectus* were evaluated. The results show that *M. oleifera* seed extract is rich in Hesperidin, Quinic acid, gallic acid, protocatechuic acid, 4-OH benzoic acid, cynaroside, isoquercitrin, cosmosiin, quercitrin, luteolin, naringenin, salicylic acid, Apigenin and Fumaric acid. The acetone extract, from day 2, showed a 100% mortality rate in all doses compared to about only 14% in the control treatment. The number of eggs laid in control (236.67) was higher than the null (0) in acetone treatment at 50 µl/ml. *M. oleifera* seed extracts contain chemical molecules. They significantly reduced the damage caused by *A. obtectus* on stored *P. vulgaris* grains. Therefore, they can be used as an alternative to chemicals for the protection of stored foodstuffs.

## 1. INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is a food crop originating from Central and South America (Chacón et al., 2005). It is rich in starch, protein and plays an essential role in the human diet, especially in some tropical regions. Its high protein content makes it one of the most important food crops for people in the South (Blair et al., 2006; Broughton et al., 2003). Globally, the common bean is ranked as the most essential consumed food crop, with an estimated annual production of 26,902,672 tons on an area of 33,066,183 hectares in 2019 (Anonymous, 2019).

Cameroon is the second most cultivated leguminous after groundnuts, with a national estimated production of 413,723 tons on 307,020 hectares (Anonymous, 2019). The Western highland is the central production zone with a production of 284,676 tons on an area of 183,592 hectares in 2016 (Anonymous, 2017). Beans belong to the group of crops capable of fixing and using atmospheric nitrogen thanks to the rhizobium located in the nodules (Doucet, 1992).

However, storage pests and pathogens can attack these grains and cause quantitative and qualitative damages such

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as loss of weight and nutritional value, contamination by mycotoxins, and production of off-odours (Baoua et al., 2015; Czembor et al., 2015). Insect pests are therefore the major constraint to seed storage, and among these storage pests of common beans, *Acanthoscelides obtectus* (Say) (Coleoptera: Chrysomelidae: Bruchinae), is a cosmopolitan pest of stored legumes that primarily utilizes the common bean (*P. vulgaris* L.). Some studies have suggested that the beans annual yields can suffer a loss of 40% if infected storages are untreated (Savkovic´ et al., 2019) . These losses go far above the recommended economic threshold of 4% (Hagstrum & W, 2014)

In order to solve these problems and ensure food security, many countries are using synthetic pesticides. Though effective and easy to use, their intensive and uncontrollable use has many drawbacks (Salim, 2011). These include; the appearance of resistant insect strains, consumer poisoning and environmental pollution (Belkebir, 2018; Guèye et al., 2011). Faced with these nuisances, the need for friendly alternatives to human health and the environment is necessary. Therefore, plant extracts could present a solution in regulating insect pests of grains stocks (Oliveira et al., 2020; Shah et al., 2017; Sherin, 2018; Sujatha et al., 2012). The antiparasitic activity of *Moringa oleifera* seeds has already been the subject of numerous studies, at the end of which it has been identified with fungicidal (Ayirezang et al., 2020), bactericidal properties (Valarmathy et al., 2010), insecticidal properties (Ezeaku et al., 2015; Oliveira et al., 2020; Shah et al., 2017; Sujatha et al., 2012). Given the biopesticide character of this plant, this study aimed to evaluate the chemical characterization and insecticidal effect of *M. oleifera* seed extracts on the developmental stages of *A. obtectus* in stored beans at different stages of its development.

## 2. MATERIAL AND METHODS

### 2.1. Material

#### 2.1.1 Plant material

The plant materials used were seeds of *M. oleifera* obtained in the mokolo market situated in Yaoundé. The common "MEX 142" bean variety (small white bean) was obtained from the Institute of Agricultural Research for Development (IRAD).

#### 2.1.2 Insect material

The insect populations of *A. obtectus* were reared in Polystyrene buckets under optimal conditions for their development (temperature 27 °C; relative humidity 75% for 15 days) following the method described by Damerdji and Bouklikha (2009).

#### 2.1.3 Chemical material

The chemical material used in the experiment consisted of: Three extraction solvents: pure Acetone, Ethanol 90° and Methanol (100% pure alcohol); and Chemical insecticide: SINOGRAIN 2% Dp with 20 g of pyrimiphos methyl as the active ingredient.

### 2.2. Methods

#### 2.2.1 Preparation of organic extract

Mature *M. oleifera* seeds were previously dried at room temperature for seven days in the laboratory. These seeds were then weighed and ground to obtain the powder. 500 g of seed powder were weighed and macerated in 2 litres of organic solvent and incubated for 72 hours (Stoll, 1994). The whole solvent + solute was filtered using a filter paper, and the filtrate obtained was concentrated with a rotary evaporator. The concentrated extracts obtained were stored in a refrigerator at 4°C until use.

#### 2.2.2 Phytochemical analysis

The instrument system used for the quantitative screening of 53 phytochemicals in the studied extracts was a Shimadzu brand LC-MS-8040 model tandem mass spectrometer coupled with a Nexera model Ultra-High Performance Liquid Chromatography (U-HPLC). Binary pumps (LC 30AD), a column oven (CTO 10 ASvp), an autosampler (SIL 30 AC) and a degasser (DGU 20 A3R) were the components of the separation system (the chromatography). The analyses were performed by a previously developed and validated LC-MS/MS method (Yilmaz, 2020). The optimized chromatographic conditions were as follows: Agilent-Poroshell 120 EC-C18 (150 mm × 2.1 mm, 2.7 $\mu$m) reversed-phase analytical column was used, LC column temperature was 40°C, eluent A (water/5 mM ammonium formate/0.1% formic acid) and eluent B (methanol/5 mM ammonium formate/0.1% formic acid) were the components of gradient elution. The gradient elution program was 0–25 min (20–100% B), 25–35 min (100% B), 35–45 min (20% B). Moreover, the flow rate of the solvent and the injection volume were optimized as 0.5 mL/min and 5 $\mu$L, respectively.

A Shimadzu LCMS-8040 model tandem mass spectrometer was utilized to accomplish the mass spectrometric detection. In addition, Lab Solutions (Shimadzu) was the software used to process the acquired LC-ESI-MS/MS data. The MRM (multiple reaction monitoring) modes were used for the quantification of the phytochemicals. The MRM method was optimized to selectively detect and quantify phytochemical compounds based on screening specified precursor phytochemical-to-fragment ion transitions. Nebulizer gas (Nitrogen) flow, drying gas (Nitrogen) flow, heat block temperature, DL temperature, and interface temperature were optimized as 3 L/min, 15 L/min, 400°C, 250 °C and 350 °C, respectively (Yilmaz, 2020). The detailed analytical parameters of the applied validated method were given in Appendix A (**Table S1**).

#### 2.2.3 Insecticidal activity

2.2.3.1 **Collection and rearing of insects** Mass rearing was carried out on healthy common bean seeds (large red bean with white spots, black and white). After twenty days (time required for perfect oviposition), the rearing medium was sieved to remove all live and dead insects. The rest is then kept under the same

conditions to allow a new generation of adult insects.

2.2.3.2 **In vivo evaluation of the insecticidal activity of the extracts** *In vivo* evaluation of the insecticidal effect of extracts was done at doses of 12.5, 25 and 50 µl/ml each. Extracts were obtained by diluting the volumes of crude extracts in 1 ml of ethanol. In each jar, 15 A. obtectus adults were introduced in transparent polystyrene jars containing pre-weighed 50 common bean seeds of the MEX 142 variety. To these, different doses of *M. oleifera* seed extracts were added. The negative control jars, prepared under the same conditions, were treated only with ethanol (1 ml). A synthetic insecticide, SINOGRAIN 2% DP (20 g pyrimiphos methyl as the active ingredient), commonly used to control insect pests of stored grains, was used as a positive control. The jars were arranged and stored randomly at a photoperiod of 12/12 h.

The mortality rate was calculated by the following formula (Singh & Jakhmola, 2011):

Mortality rate = (Number of dead insects / Total number of insects) x100

2.2.3.3 **Assessment of extract activity on egg-laying and emergence** The total number of laid eggs and the percentage of hatched eggs were counted. After the death of the insects introduced in different jars, the bean seeds were observed with a magnifying glass to count the eggs laid by the insects before their death. These seeds were then reintroduced into the same jars and kept until the new generation (F1) appeared. Once they had appeared, they were also counted. This allowed us to calculate the emergence rate following the formula used by Singh and Jakhmola (2011):

ER = ((AC-AT) / AC) x 100

Where ER = Emergence rate; AC = Number of adults emerged in control jars; AT = Number of adults emerged in the treated jars

### 2.3. Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA) using SPSS version 16.0 software. The multiple comparisons of means were made at the 5% threshold using the Duncan MRT (Multiple Range Test).

## 3. RESULTS

### 3.1. Phytochemical analysis

The data collected of the three extracts' chemical composition and the concentration of specific molecules were presented in **Table 1**. It was noted that extraction solvent affected the chemical composition of extracts. For instance, 12 and 13 molecules were found in the methanol and ethanol extract, respectively. Remarkably, of the 53 molecules, none was found in the acetone extract. Fumaric acid and 4-OH benzoic acid levels were highest in the methanol extract (1.263 and 0.570, respectively), suggesting that methanol might be more suitable for obtaining fumaric acid, quinic acid and 4-OH benzoic acid.

### 3.2. Effect of *M. oleifera* seed extracts on the mortality rate of *A. obtectus* adults

The survival of *A. obtectus* adults on the application of *M. oleifera* seed extracts is presented in **Table 2**. In general, adults of A. obtectus can survive for at least four days. However, the mortality rate was more pronounced in batches treated with *M. oleifera* extracts. The analysis of the mortality rate shows significant differences ($P < 0.05$) between the treatments at the same concentrations. The increase in the mortality rate of treatment varies proportionally with the increase in concentration.

Methanol extract showed a higher mortality rate of 88.78%, 93.8% and 96.55% for the concentrations 12.5, 25 and 50 µl/ml respectively on day 1. Contrarily, a low mortality rate of 7.80%, 13.94% and 60.6% was recorded in control on days 1, 2 and 3, respectively. Also, treatment with chemical insecticide had a mortality rate of 100% from the first day of treatment.

### 3.3. Effect of *M. oleifera* seed extracts on the egg-laying and emergence rate of *A. obtectus* adults

Data in **Table 3** shows the number of eggs laid by *A. obtectus* adults and the emergence rate of the latter. In general, the different Moringa seed extracts significantly reduced egg-laying in *A. obtectus* adults and the emergence rate of *A. obtectus* lava ($P < 0.05$) compared to the negative control. However, the number of eggs laid decreased with an increased concentration of extract used. These rates vary from 236.67 % in control to 0.00 % in the acetone extract treatment at a dose of C3 (EA3). On the other hand, there was no significant difference between the results obtained with insecticidal treatment and the treatment with AE3, where no eggs were laid.

As concerns emergence rate, it decreased with an increase in the concentration of extracts used. Generally, no significant difference was observed in the control treatment (87.76 %), methanol extracts at a dose of C1 (ME1: 84.66 %) and ethanol extract at a dose of C1 (EE1: 84.39 %). Also, no significant difference was observed between treatments with insecticide and acetone at a dose of C3 (AE3), where no emerged insects were recorded.

## 4. DISCUSSION

Three hundred gram of *M. oleifera* seeds extracted with methanol yielded 15.5 %, 19.1 % with acetone and 16.6 % with ethanol. The yield difference between the organic solvents is due to the solubility of the compounds, which depend on the properties of the solvent (Muhammad et al., 2013). Furthermore, the high polarity of organic solvents (methanol, ethanol, and acetone) would bind to many compounds in *M. oleifera* seeds, thereby increasing the extraction yield (Muhammad et al., 2013).

The toxicity results of *M. oleifera* seed extracts on Acanthoscelides obtectus show that its extracts influenced adult survival. This could be explained by the presence of secondary compounds in the extracts, such as Salicylic acid, Quinic acid,

**Table 1**
Chemical composition of the tested extracts (mg/g extract)

| Chemical compounds | Methanol | Acetone | Ethanol | Chemical class | Biological activity | Reference |
|---|---|---|---|---|---|---|
| Hesperidin | 0.006 | nd | 0.009 | Flavonoid | Fungicide and insecticide | Ilboudo et al. (2016); Kopustinskiene et al. (2020) |
| Quinic acid | 0.933 | nd | 0.297 | Phénolic acid | Insecticide | El-Kady et al. (2010) |
| Fumaric acid | 1.263 | nd | 0.153 | Organic acid | Nd | Nd |
| Gallic acid | 0.014 | nd | 0.013 | Phénolic acid | Fungicide | Dang-Minh-Chanh et al. (2013) |
| Protocatechuic acid | 0.051 | nd | 0.020 | Phénolic acid | Fungicide | Nguyen et al. (2014) |
| 4-OH Benzoic acid | 0.570 | nd | 0.207 | Phénolic acid | Nd | Nd |
| Cynaroside | nd | nd | 0.012 | Flavone | Fungicide | Amoroso et al. (2021) |
| isoquercitrin | 0.037 | nd | 0.017 | Flavonol | Fungicide | Buśko et al. (2014) |
| Cosmosiin | nd | nd | 0.003 | Flavonoid | Nd | Nd |
| Quercitrin | 0.089 | nd | 0.077 | Flavonol | Fungicide | Buśko et al. (2014) |
| Luteolin | 0.003 | nd | 0.002 | Flavone | Fungicide | Buśko et al. (2014) |
| Naringenin | 0.002 | nd | nd | Flavonoid | Fungicide | Buśko et al. (2014) |
| Apigenin | 0.001 | nd | 0.001 | Flavonoid | Fungicide | Buśko et al. (2014) |
| Salicylic acid | 0.030 | nd | 0.010 | Phénolic acid | Fungicide, insecticide, growth hormoneand stimulator of the natural defenses of the plant. | Dieryckx et al. (2015); Ola (2016) |

nd: not détermined

**Table 2**
Effect of *Moringa oleifera* seed extracts on the mortality rate of *Acanthoscelides obtectus* adults

| | Mortality rate per day (%) | | |
|---|---|---|---|
| **Treatments** | **Day 1** | **Day 2** | **Day 3** |
| Control | $7.80^{h} \pm 1.25$ | $13.94^{d} \pm 0.76$ | $60.6^{d} \pm 2.91$ |
| ME1 | $88.78^{e} \pm 1.07$ | $94.43^{b} \pm 1.46$ | $100^{a} \pm 00$ |
| ME2 | $93.8^{c} \pm 1.08$ | $100^{a} \pm 00$ | $100^{a} \pm 00$ |
| ME3 | $96.55^{b} \pm 0.35$ | $100^{a} \pm 00$ | $100^{a} \pm 00$ |
| EE1 | $82.07^{g} \pm 1.92$ | $86.17^{c} \pm 0.84$ | $93.33^{c} \pm 00$ |
| EE2 | $86.83^{f} \pm 0.47$ | $93.88^{b} \pm 0.96$ | $95.33^{b} \pm 1.73$ |
| EE3 | $90.87^{d} \pm 0.31$ | $97.77^{b} \pm 0.35$ | $100^{a} \pm 00$ |
| AE1 | $88.22^{ef} \pm 0.19$ | $100^{a} \pm 00$ | $100^{a} \pm 00$ |
| AE2 | $91.11^{d} \pm 1.92$ | $100^{a} \pm 00$ | $100^{a} \pm 00$ |
| AE3 | $96.37^{b} \pm 0.19$ | $100^{a} \pm 00$ | $100^{a} \pm 00$ |
| Chemical insecticide | $100^{a} \pm 00$ | $100^{a} \pm 00$ | $100^{a} \pm 00$ |
| **Means** | **$83.85^{a} \pm 25.72$** | **$91.67^{a} \pm 25.83$** | **$95.39^{a} \pm 11.76$** |

The same letter markings indicate insignificant differences, and markings with different letters have significant differences at the level of $p \leq 0.05$ (Duncan test). ME: methanol extract; EE: ethanol extract; AE: acetone extract. 1: concentration 1 (12.5 $\mu$l/ml); 2: concentration 2 (25 $\mu$l/ml) and 3: concentration 3 (50 $\mu$l/ml)

**Table 3**
Variation in the number of eggs laid by *Acanthoscelides obtectus* adults and their viability rate according to the different treatments

| Treatments | Number of eggs laid | Emergence rate (%) |
|---|---|---|
| Control | $236.67^{a} \pm 9.6$ | $87.76^{a} \pm 4.7$ |
| ME1 | $56.67^{c} \pm 3.05$ | $84.66^{a} \pm 2.48$ |
| ME2 | $23^{e} \pm 2.64$ | $73.62^{ab} \pm 6.02$ |
| ME3 | $2.66^{g} \pm 0.57$ | $17.77^{c} \pm 13.47$ |
| EE1 | $79^{b} \pm 3.6$ | $84.39^{a} \pm 1.09$ |
| EE2 | $35.667^{d} \pm 2.52$ | $80.27^{ab} \pm 3.66$ |
| EE3 | $9.33^{f} \pm 2.31$ | $65.28^{b} \pm 16.83$ |
| AE1 | $51.67^{c} \pm 1.53$ | $82.03^{ab} \pm 5.72$ |
| AE2 | $14.33^{f} \pm 3.21$ | $79.34^{ab} \pm 3.48$ |
| AE3 | $0^{g} \pm 00$ | - |
| Chemical insecticide | $0^{g} \pm 00$ | - |

The same letter markings indicate insignificant differences, and markings with different letters have significant differences at the level of $p \leq 0.05$ (Duncan test). ME: methanol extract; EE: ethanol extract; AE: acetone extract. 1: concentration 1 (12.5 $\mu$l/ml); 2: concentration 2 (25 $\mu$l/ml) and 3: concentration 3 (50 $\mu$l/ml)

Hesperidin, Fumaric acid etc. According to Boulogne and Sciences du Vivant [q-bio] (2011), almost 116 molecules are identified to have insecticidal activity in plant extracts and the molecules most often responsible for this are terpenoids, alkaloids and phenolic compounds. However, the insecticide activity of organic extracts of *M. oleifera* is due to the biological activity of the compounds present in these extracts, which have an anti-nutritional effect and cause respiratory disorders. They inhibit nutrition and cause death and malformations in future generations of phytophagous insects (Carpinella et al., 2003). The results of this work are similar to those of Oliveira et al. (2020). They showed that phytochemical analysis of water extract (WE) from *M. oleifera* seeds and water-soluble lectin (WSMoL) revealed the presence of flavonoids, tannins, saponins, phenylpropanoids, alkaloids, and reducing sugars in the WE. After application of artificial diet composed of wheat flour supplemented with WE (60.0-640.0 mg/g) or WSMoL (0.5-60 mg/g) the extract was toxic to S. zeamais ($LC_{50}$: 214.6 mg/g) even if WSMoL caused slight mortality (12.0 $\pm$ 2.7%) at the highest tested dose (60 mg/g). Also Ibrahim and Aliyu. (2014) Ibrahim and Aliyu (2014) showed that the lowest mean number of holes was recorded in cowpea seeds treated with African nutmeg oil and moringa seed oil. Moreover, Ouedraogo et al. (2016) showed that *Ocimum gratissimum* oil at the dose of 75 $\mu$l causes 80% mortality of *Sitophilus zeamais* adults within 24h compared to 99.5% when 100$\mu$l of *Cimbopogon nardus* essential oil is used for 72h. After the application of 50$\mu$l of O. gratissimum oil for 48 h on the adults of *Rhyzopertha dominica*, the mortality rate was 100%.

The insects were able to lay eggs during the experimental period. This was observed from the presence of eggs in control and some treated jars. However, the significant differences (P < 5%) observed between the treated and control jars showed that the extracts influenced the laying of eggs. This result can be explained because the seed extracts' toxicity at these doses would have prevented the adults from laying eggs before dying. This phenomenon is confirmed by the emergence rate where a low appearance of individuals was observed in the treated batches compared to the untreated batches. This result could be explained by the fact that the extracts caused the insects' early death, reducing egg-laying by the females or the non-viability of the eggs laid. The results are similar to those of Sherin (2018), who obtained 84.64% of oviposition in the control batches with little or no oviposition treated with *M. oleifera*, *Simmondsia chinensis* and *Prunus dulcis* oil at 25, 30 and 35% concentrations.

In the presence of Moringa extract, the emergence of F1 individuals of *A. obtectus* showed a decrease in the number of emerged individuals with an increase in the doses of Moringa oil applied. The maximum reduction was observed at a dose of 50$\mu$l/ml. This decrease in emerged individuals could be due to a reduction in gas exchange between the *A. obtectus* larva and the medium, increasing with the concentration. These results agree with those of Wahedi et al. (2013) who showed that neem seed extract significantly prevented the emergence of F1 adults of C. maculatus, and no subsequent weight loss was made due to pests. Moreover, Sherin (2018) obtained a maximum reduction (100%) in the emergence of *C. maculatus* F1 for concentrations of 25, 30 and 35% of Jojoba, Moringa, Fenugreek and sweet almond oils. Woguem (2017) obtained complete inhibition of F1 emergence of *A. obtectus* with a dose of 1.6$\mu$l/g of *Mondia whitei* essential oil and 0.64$\mu$l/g of *Echinops giganteus* essential oil.

## 5. CONCLUSION

This work has demonstrated the biopesticidal potential of *M. oleifera* extracts against *A. obtectus* in stored *P. vulgaris*. It also presented the substances present in the extracts of *M. oleifera* seeds that could be responsible for its biopesticidal activity and their effect on the viability and multiplication of *A. obtectus* in natural conditions. However, the results obtained show that the substances contained in *M. oleifera* seed extracts are likely to reduce the infestation of common bean seeds in storage by the pest *A. obtectus*. Therefore, it is clear that using *M. oleifera* seeds from 25 $\mu$l/ml in the control of *A. obtectus* is an alternative to chemical control.

## CONFLICTS OF INTEREST

Given his role as Associate Editor, Gokhan Zengin has not been involved and has no access to information regarding the peer review of this article. Full responsibility for the editorial process for this article was delegated to Co-Editor Carlos L. Cespedes Acuña. There is no conflict of interest among the authors or any other person.

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## A. APPENDIX. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at ht tps://doi.org/10.53365/nrfhh/143056.

## AUTHOR CONTRIBUTIONS

TAS, NB - Research concept and design, WNKT - Collection and/or assembly of data, WNKT, DMA, MAY, KIS - Data analysis and interpretation, DMA, PNZ, MAY, KIS - Writing the article, PNZ, GZ, ZA - Critical revision of the article, GZ, ZA - Final approval of the article.

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