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EFFECTIVENESS OF NEEM SEED EXTRACTS IN THE FIGHT AGAINST WEEVILS OF MAIZE IN STORAGE FOR SUSTAINABLE EATING PRACTICES

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Mots clés

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toxicity test,

IMPM

Abstract

Context: The invasion of maize pests (*Sitophilus zea mays* Motschulsky) in crops and storage areas causes enormous losses. To overcome this constraint, producers and owners of storage warehouses generally use synthetic insecticides. These are polluting, expensive and have harmful effects on the health of consumers and the environment. In the search for alternatives for healthy and sustainable eating practices, the objectives of this work were to evaluate the repellent capacity of *Azadirachta indica* seed oil extract on corn weevils in storage, its efficacy on maize weevils in storage, and the contact efficacy of seed powder extracts on maize weevils in storage

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Methods: Phytochemical screening was carried out on the extracts obtained according to some known procedures. The effectiveness of Neem extracts was determined by repellency; contact toxicity tests were carried out according to the usual methods. **Results:** Phytochemical screening shows secondary metabolites such as terpenes, flavonoids, phenols, alkaloids, tannins, Anthraquinones, coumarins, Anthocyanins, terpenes, among which you can find the actives principles of A. *indica*, responsible of the insecticide activity of Neem extracts. Neem oil is repellent at 62.5 %. This allows us to categorize it in the class IV of repulsive substances. However, it's moderately repellent during the first two hours and repellent after two hours. This oil was lethal against *Sitophilus Zea mays,* with 100 % mortality by contact at a dose of 400 µl, and a nearby result at 200 µl. Neem powder caused 100 % mortality from the third day, after the treatment at most of doses (LC_{50} = 94,62 µl, LC_{50} = 1,07 g in 24h). **Conclusion:** Our Neem extracts protect the production against weevils and avoids losses, ensuring food security.

I-INTRODUCTION

CONTEXT:

Goals number five and three of the African Union's Agenda 2063, recommend modern agriculture for increased productivity and production, good health and good nutrition for citizens also. They are in line with the second Sustainable Development Goal (SDG 2), which recommends ending hunger, ensuring food security, improving nutrition, and promoting sustainable development. According to Cameroon's national development strategy (SND30), in 2017, 16% of households suffered from food insecurity in urban areas and 22.2 % in rural areas (Anonymous 1, 2020). This situation is similar or much more alarming in others areas of the country or continent and is getting worse with the demographic boom. At a time when the importation of wheat and rice is experiencing problems due to the Russia-Ukraine war, independently of Africa. Maize which was already the most consumed cereal in Africa, is now proving to be an important solution to the food problem affecting the continent. Maize production, like other local crops, should therefore increase and be protected in quantity and quality. In fact, this production faces threats including insect pests such as corn stalk and ear borers, leaf insect pests of corn, and especially insect pests of corn in storage such as Maize weevils (*Sitophilus Zea mays* , *Sitophilus oryzae*) the greater maize borer, the beetles and the grain aluce (Sikirou et *al*, 2018). Damage caused by Sitophilus is estimated at around 20-25 % of field production, 15-20 % of post-harvest losses. Total losses would be estimated at 35 to 45 % of the food crop value chain in Cameroon. (IRAM & MINADER, 2017).

The fight against the pests mentioned above is done by traditional methods and technics including exposure to the sun, smoking, the use of repellent or insecticide plants such as pepper, the use of inert materials, some ashes, conservation in a confined atmosphere (Anonymous 3, 2018). Biological control uses antagonistic living organisms, predators of pests such as nematodes, arthropods, as well as parasitoids, corn borer, wasps *sympiesis virudila*, *Macrocentrus cingulum*), tachin fly *Lydella thompsoni*, Parasitoids of aphids, Wasps aphidin , etc. (Anonymous 2, 2014; Christine & Josée , 2009).

This fight against pests of crops and harvests is mainly done through synthetic insecticides, approved or not. Among them: organochlorines, whose active ingredients can be Dichloro-Diphenyl-trichloroethane (DDT) and other chlorinated derivatives; organophosphates, the active ingredients of which may be malathion, phosmet, pirimiphos-methyl, pirimiphos-ethyl, parathion-methyl, etc. ; carbamates having among others as active ingredients: bendiocarb, carbarl, methi ocarb, etc. insecticides from the pyrethroid family may have as active ingredients: acrinathrin, allethrin, bromethrin, etc**.** neonicotinoids include clothianidin, dinotefuran, imidacloprid, etc. (Anonymous 2, 2014). However, according to the World Health Organization (WHO), few years ago, synthetic pesticides are the cause of the death of 200,000 people each year around the world, and in Africa these risks are increased. The United Nations Environment Program (UNEP) also notes that in sub-Saharan Africa, the potential cost of treating diseases linked to pesticides between 2005 and 2009 was estimated at 6.3 billion; and that of 2005 and 2020 was estimated at 90 billion dollars (Aggrey, 2017; Anjarwalla *et al*, 2016). On August 26th 2024, all the Cameroonians' medias have reported on a tragedy where six persons died after eating maize crops which had been treated with synthetic pesticides.

For a long time, there was no comprehensive scientific study aimed at beetle insects such as S. Zea mays. This is due to the fact that it is in tropical countries and particularly in Africa, where research was not developed that these insects are big threats, because the climate of western and rich countries is not favorable to them. Note, however, the studies by Tamgno & Ngamo (2014) who worked on the use of Neeem (*A. indica*) as alternative to synthetic insecticides for the protection of maize and sorghum seeds in the logone valley. Diabate *et al*. (2020), worked on the toxicity of aqueous extracts of *A. indica* and *Jatropha curcas* on *Plutella Xylostella* by contact. Furthermore, the Ministries of Agriculture of certain West African countries such as Benin, have recommended the informal use of *A. indica* extracts to farmers as a Biopesticide (Sikirou *et al*., 2020). In Cameroon, there is almost no usable data on this subject. Our study provides additional scientific informations to that field. This will convince skeptics and provide assurance for the effective use of *A. indica* as a Bioinsecticide.

We face a double problem which is that of the invasion of insect pests of crops, and storage areas such as maize weevils, as well as damage to the health of consumers and the environment, due to synthetics pesticides or insecticides used in the fight against harmful organisms. The urgency of the development of natural insecticides or plant-based Bioinsecticides is signaled and proves to be a strategic element in the quest for large production, food security and environmental protection.

The question is to know how can we effectively control pests such as weevils which affect plants and stored foodstuffs such as maize, without polluting the environment or harming the health of consumers? And the research hypothesis we are working on is that: the toxic effects of *Azadirachta indica* (Neem)-based bio-insecticide extracts can effectively control corn weevils, while helping to reduce environmental pollution by replacing the synthetic insecticides used. So, our **general objective** is "to study the toxic effects of oil and powder extracts from the seeds of *A. indica* on *S. Zea mays* (Coleoptera: Curculionidae) of maize in storage. The **specifics objectives** were to evaluate the repellent capacity of *Azadirachta indica* seed oil extract on corn weevils in storage, its efficacy on maize weevils in storage, and the contact efficacy of seed powder extracts on maize weevils in storage

II-MATERIALS AND METHODS

II.1. Material

II.1.1. ***Study site***

The main site of the study is the Center for Research on Medicinal Plants and Traditional Medicine of the Institute of Medical Research and Medicinal Plants studies (CRPMT-IMPM) of Ngoa-ékelle in Yaounde. Samples of *A. indica* were collected at Mokolo market in Yaoundé (Cameroon) and *S. Zea mays* at Mfoundi market. The manipulations at the CRPMT-IMPM were carried out in particular in the laboratory of botany and traditional medicine for the repulsion and contacts assays of the *A. indica extracts* on *S. Zea mays* and the laboratory of Galenics and Pharmaceutical Technology for the pressed oil sample. machine. The experiment was done in ambient average temperature of about 25 ° C.

II.1.2. ***Biological material.***

The animal material used was *S. Zea mays*, and the plant material, included *A. indica* s and *Zea mays* seeds (figure 1). Samples of *S. zea mays* which are of the genus Sitophilus, of the order of Coleoptera and of the family of Curculionidae were collected at the Mfoundi market in Yaoundé, in untreated maize waste (stalks, bran), infested and sorted before the sale.

The shelled kernels and seeds of *A. indica* A. juss, were purchased at the Mokolo market in Yaoundé, from the northern part of Cameroon.

The North Cameroon white maize seeds used were purchased at the Mfoundi market in Yaoundé.

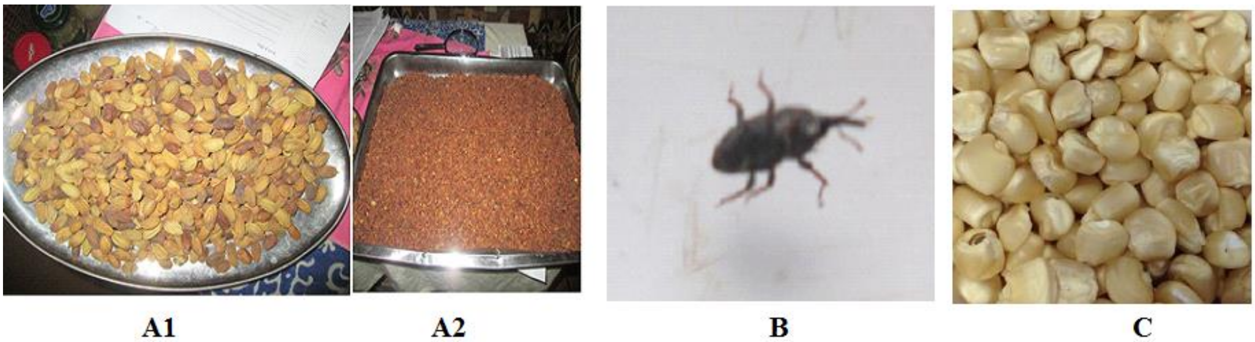

Figure 1: *Biological Material A1&A2: Seeds and Kernels of Azadirachta indica; B: Sitophilus Zeamais (Scale: 5); C: Maize in storage.*

II.1.3. Equipment and others.

Equipment used:

The manual crushing machine, the manual winepress machine, 1-liter glass jars, venti-line brand oven, syringes, filter paper, petri dishes, glass jars, gangs, sampling tubes, sensitive balance, camera, cotton, acetone, etc.

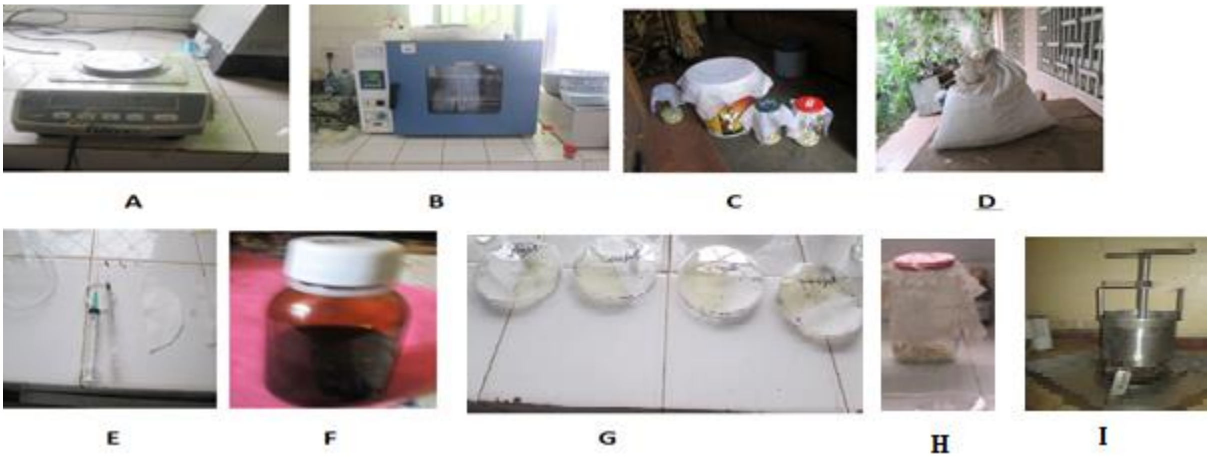

Figure 2. *Equipment. A: sensitive equilibrium; B: Oven; C et D: Sitophilus zeamais rearing device; E: syringes; F: Neem oil; G: petri dishes; H: glass jar; I: oil press*

II.2. Methods

This study is an experimental study, carried out in the laboratories mention above.

II.2.1. *Collection and breeding of corn weevils.*

The samples of *S. zeamais* were collected from a maize seller's store at the Mfoundi market in Yaoundé. They were collected with cob debris and maize bran containing a few handfuls of seeds, in a bag (Nyetam, 2022). Subsequently, they were transposed into a bucket covered with a transparent fabric, then into glass jars, as they approached use. Breeding was therefore done in mass.

II.2.2. *Choice and packaging of the maize variety.*

The variety chosen is white maize from North Cameroon (large and small grains) found in our markets, for breeding and experimentation. Before the experiment, we stored our seeds in clean glass jars. They were put in the refrigerator three days before the experiment in order to kill the germs, then removed and dried (Boeke *et al.*, 2001).

II.2.3. *Preparation of Azadirachta indica seed extracts*.

The almonds previously dried at ambient temperature are put in an oven at 60 °C, during twelve hours. A quantity of 843 g of *A. indica* seed almond powder is obtained after molding 860 g of dried seed almonds using a manual mill, shortly before the extraction process and shortly before the various necessary tests requiring the powder (Nyetam, 2022).

800 g of crushed *A. indica* seed powder are introduced into a makeshift cylindrical press with a grid and cotton at its base, then pressed so as to drop its oil into a container placed below.

$$\textbf{Efficiency}\ (\%\ ) = \frac{(\textbf{Mass of extract (g)}}{\textbf{Mass of plant material (g)}} X\textbf{100}$$

II.2.4. *Effectiveness of A. indica seed extracts on weevils.*

The repulsion test or extract avoidance test is used to determine whether weevils, given the choice, are able to detect the extract from *A. indica* seeds and sidestep it.

The repellency assay is used to calculate the percent repellency of seed oil on corn weevils by the preferential zone method described by Mc Donald et *al*. (1970). Indeed, the discs of whatman filter paper n° 2 of 9 cm in diameter were divided into two equal parts of 31.79 cm^2 of surface (Fig. 3). Four concentrations were prepared by dilution in 0.5 ml acetone, of the following seed oil volumes: 100, 200, 300 and 400 µl. The solutions thus obtained were homogenized by manual stirring and produced on 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 were then allowed to elapse, the time for the solvent to evaporate completely. The following oil concentrations were thus obtained (volume/area): 3.14 µl/cm^2, 6.29 µl /cm^2, 9.43 µl /cm^2 and 12.58 µl /cm^2. The filter paper discs were reconstituted depending on the treated halves and the control halves using clear adhesive tape and placed in petri dishes (fig.3). Fifteen adult weevils, just taken from their rearing medium, were placed in the center of each paper, and the petri dishes covered. Four replicates were performed for each oil concentration. The counting of weevils on each half of the disc was carried out after 1 hour, 2 hours, 3 hours and 4 hours, of treatment under laboratory conditions. The percentage repellency is calculated according to the formula used by Nerio *et al.* (2009) as follows:

$$\mathbf{PR}(\%) = \frac{(\mathbf{Nac} - \mathbf{Nh})}{(\mathbf{Nac} + \mathbf{Nh})} X100$$

PR= Percentage of repulsion

Nac= number of weevils present on the half-disc treated with acetone.

Nh= number of weevils present on the half-disc treated with the dose of oil.

Figure 3 : *Repellency test.*

Average repellency percentage is calculated and assigned to the different repellent classes according to the classification of Mc Donald *et al* (1970), represented by the following Table I:

Table I. Repellent classes according to the classification of Mc Donald *et al* (1970).

Repellent classes	Repulsion intervals (%)	Properties
Class 0	< 0,1	Not repellent
Class I	0,1-20	Very weak repellent
Class II	20,1-40	Weakly repellent
Class III	40,1-60	Moderately repellent
Class IV	60,1-80	Repellent
Class V	80,1-100	Very repellent

Mc Donald et *al.*, 1970.

Volumes of 100, 200, 300, 400 µl of *A. indica* oil are prepared by dilution with 1ml of acetone and mixed with 30 g of corn contained in 1000 ml glass jars. The whole was homogenized by manual stirring and left for 5 to 10 minutes for complete evaporation of the solvent. The respective concentrations of 3.33; 6.66; 9.99 and 13.33 µl/g were obtained. Then, 15 adult insects were introduced into each jar, and the latter was covered with porous fabric held in place with elastic ties. The control dishes were prepared under the same conditions and treated only with acetone (1ml). Four tests of 15, 15, 10, 10, insects, are carried out per dose and for the control, the results expressed for an average of 25 insects. The jars are stored under ambient conditions in the laboratory.

Fifteen adult maize weevils are introduced into transparent polystyrene jars containing 30 g of white maize seeds. These are treated with different doses of *A. indica* seed powder.

Four doses of *A. indica* seed powder (1, 5, 10, 15 g) are used for 30 g of maize seeds; i.e. 3.33 respectively; 16.67; 33.33 and 50 % of the weight of the seeds, control batches (0%) are carried out in parallel with untreated seeds. Four repetitions are carried out for all the doses and for the control. The jars are stored under ambient conditions in the laboratory.

Dead insects were counted every 24 hours during a period of the experiment. The mortality rate was assessed through the ratio of the number of dead insects to the number of insects initially introduced according to the following formula.

$$\boldsymbol{MR}(\%) = \frac{\mathbf{NCM}}{\mathbf{NCI}}\boldsymbol{X}\mathbf{100}$$

With, **MR (%):** Mortality rate; **NCM**: number of dead weevils; **NCI:** number of weevils introduced.

Mortality was calculated and corrected according to Abbott's formula taking into account natural mortality (Mt) observed on the control (Abott, 1925, cit. Ambela minkeng, 2018).

$$\boldsymbol{Mc} = \frac{\mathbf{Mo - Mt}}{\mathbf{100 - Mt}}\boldsymbol{x}\mathbf{100}$$

Mc: corrected mortality; **M0**: mortality of the sample tested; Mt: mortality in the untreated control.

And the LD_{50} or CL_{50} of Neem oil and powder were estimated according to the time of exposure of the insects to the different doses, using the smallest dose whose mortality rate is closer to 50 % with the rule of three or by plotting a standard curve giving the variations in mortality as a function of the increasing concentrations of the products.

II.2.5. *Statistical analysis of data*

Data were measured or calculated for each response variable at the test level (percentages of mortality, repellency, etc.). The raw data were visually explored to check for consistency, entered into MS-Excel, and then statistically analyzed according to the Mc Donald *et al.* (1970) classification. For these statistical analysis, computer software called graph pad Prism and Excel were used.

III. RESULTS

III.1. *Extraction Yield.*

With 860 g of dried almonds, we obtained 843 g of powder, i.e. a yield of 98.02 % and by introducing 800 g to the press, we had 56.5 g of oil, with a concentration of 1.3 g/ ml, i.e. a yield of 7.06 %, and a quantity of approximately 44 ml of *A. indica* oil. This yield is lower than what would have been obtained by solvent extraction, but the raw and natural character of the extract obtained could have advantages in activity and is easily assimilated to the oil extracts used by the farmers (Table II).

Table II. Extraction Yield

Excerpt name	Masses of plant material	Mass of the extract obtained	Yield
Powder	860g (almonds)	843 g	98,02 %
Pressed oil	800g (Powder)	56,5 g	7,06 %

III.2. *Effectiveness of A. indica seed extracts on weevils.*

-Depending on doses

At the dose of 200 µl (6.29 µl/cm2), the difference in the rate of repulsion is significant with the other doses; Neem oil is very repellent at 200 µl, while it is moderately repellent at doses of 100 µl (3.14 µl/cm^2), 300 µl (9.43 µl/cm^2), 400 µl (12.58 µl/cm^2). Because, we note that at 300 µl and 400 µl, its effect is no longer only repellent, but also lethal or paralyzing. the insects which venture into the part of the disc treated with *A. indica* oil, are paralyzed, unable to flee and thus find death. The overall repellency average is 62.25 %(Tables III).

Table III. Repellent effect of oil extracts from *A. indica* seeds on weevils depending on amounts

The amount of oil	Number of individuals		Average percent repellency	Effect	repellency class
	In the untreated part (Nac)	in the treated part(Nh)			
100µl (3,14µl/cm²)	80	20	60 %	Moderately repulsive	III
200µl (6,29µl/cm²)	94	6	88 %	very repulsive	V
300µl (9,43µl/cm²)	77	23	54 %	Moderately repulsive (but kills)	III
400µl (12,58µl/cm²)	74	26	48 %	Moderately repulsive(but kills°	III

-Depending on duration

No matter the concentration, the Neem oil is repellent toward *S. zeamais* after 2 hours, and Moderately repulsive during the first two hours (Table III). *A. indica* oil is Repulsive of class IV (Table IV).

Table IV. Repellent effect of oil extracts from *A. indica* seeds on weevils depending on durations.

	Duration	Average percent repellency	Repellency class	Effect
Azadirachta indica oil	**1h**	56	III	Moderately repulsive
	2h	56	III	Moderately repulsive
	3h	64	IV	Repulsive
	4h	74	IV	Repulsive

	Mean	62,5±8,54	IV	Repulsive

The contact of maize treated with different doses of *A. indica* oil, with *S. Zea mays* [Coleoptera: Curculionidae], shows that the mortality rate increases with the duration of contact and reaches its maximum on the 4th day for the 100 µl dose. From the 1st day, mortality reaches 100% at the dose of 400 µl and approaches this maximum for the doses of 200 µl and 300 µl, because there is no significant difference between their mortality rates, from the 1st to the 4th. day. The effectiveness of the 300 µl dose does not increase on the 2nd and 3rd day, compared to the 200 µl dose which proves to be slightly more effective during this period and compared to the 400µl dose, the effectiveness of which is maximum from the first 24 hours. The DL_{50} or CL_{50} decreases when the exposure time increases, and varies from 94.62 µl during o1 day to 55.11 µl when 4 days are needed to kill fifty percent of *Sitophilus zeamais* (Table V&VI).

Table V. Contact toxic effects of *Azadirachta indica* seed oil on maize weevils

Duration	**Corrected number of deaths and cumulative mortality rate as a function of dose and time**										
	0 µl	**100 µl**		**200 µl**		**300 µl**		**400 µl**		**1g** Sinograin	
		Deaths	Rate	Deaths	Rate	Deaths	Rate	Deaths	Rate	Deaths	Rate
24h	2	13,21	52,84 %	21,42	85,68 %	21,42	85,68 %	23,46	93,84 %	23,46	93,84 %
48h	2	20,40	81,6 %	22,44	89,76 %	21,42	85,68 %	23,46	93,84 %	23,46	93,84 %
72h	2	21,42	85,68 %	22,44	89,76 %	21,42	85,68 %	23,46	93,84 %	23,46	93,84 %
96h	3	22,68	90,72 %	22,68	90,72 %	22,68	90,72 %	22,68	90,72 %	22,68	90,72 %
MOY	2,25	19,62	77,71 %	22,24	88,98 %	21,73	86,94 %	23,52	93,06 %	23,52	93,06 %

±0, 5	±4,46	±0,16	±0,56	±0,02	±0,63	±0,02	±0,12	±0,01	±0,12	±0,01

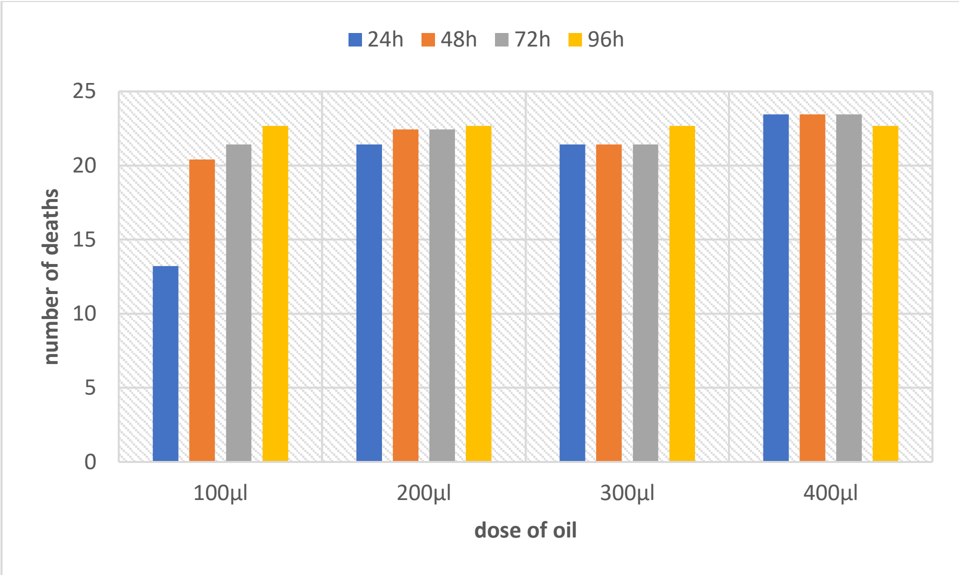

Figure 3: Corrected number of deaths as a function of dose and time

Table VI. LC_{50} of Neem Oil as function of time

	24 h	48 h	72 h	96 h
DL_{50} ou CL_{50}	3,15 µl/g	2,05 µl/g	1,9 µl/g	1,83 µl/g

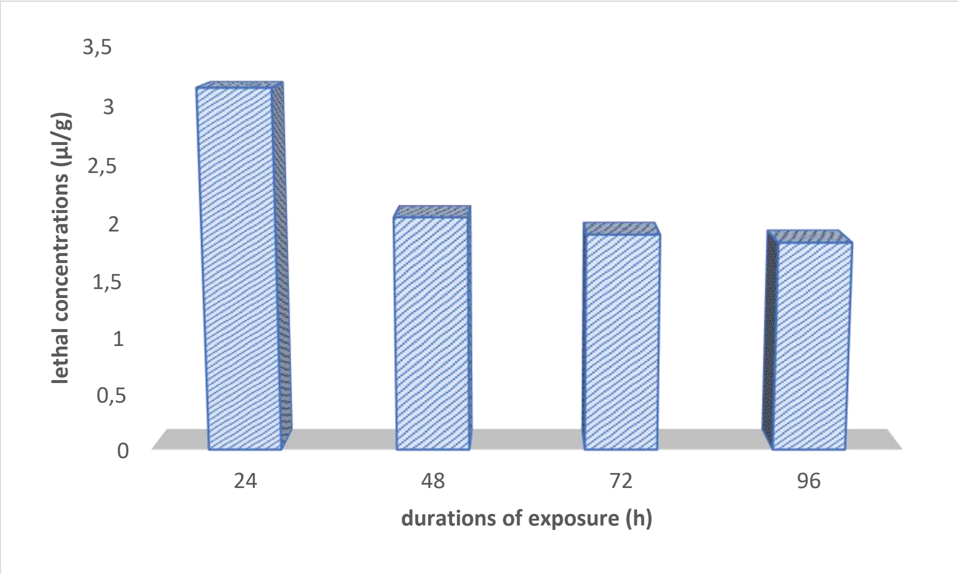

Figure 4: LC_{50} of Neem oil as function of time

The contact of maize treated with different doses of *A. indica* powder, with *S. Zea maïs* [Coleoptera: Curculionidae], shows that the mortality rate increases with the duration of contact and reaches its maximum (100 %) on the 3rd day for the dose of 5 g, 10 g and 15 g. however, the 5g dose proves to be the most effective on day 1 (93.33 % versus 46.66 %, 80 % and 86.66 %, respectively for 1 g, 10 g and 15 g). the LC_{50} varies from 1.07 g (35,66 g/kg) for 1 day to 0.53 g(17,66 g/kg) for 2, 3 or 4 days. The 5 g (166,66 g/kg) dose is the LC_{100}.

Table VII. Contact toxic effects of *Azadirachta indica* seed powder on maize weevils

Duration	Corrected number of deaths and cumulative mortality rate as a function of dose and time										
	0g	1g		5g		10g		15g		1g Sinograin	
	M	[1]M	MR	M	MR	M	MR	M	MR	M	MR
24h	0	7	46,66%	14	93,33%	12	80%	13	86,66%	15	100%
48h	0	14	93,33%	14	93,33%	12	80%	13	86,66%	15	100%
72h	0	14	93,33%	15	100%	15	100%	15	100%	15	100%
96h	0	14	93,33%	15	100%	15	100%	15	100%	15	100%

Table VIII. LC_{50} of Neem powder as function of time.

	24 h	48 h	72 h	96 h
DL_{50} ou CL_{50}	35,66 g/kg	17,66 g/kg	17,66 g/kg	17,66 g/kg

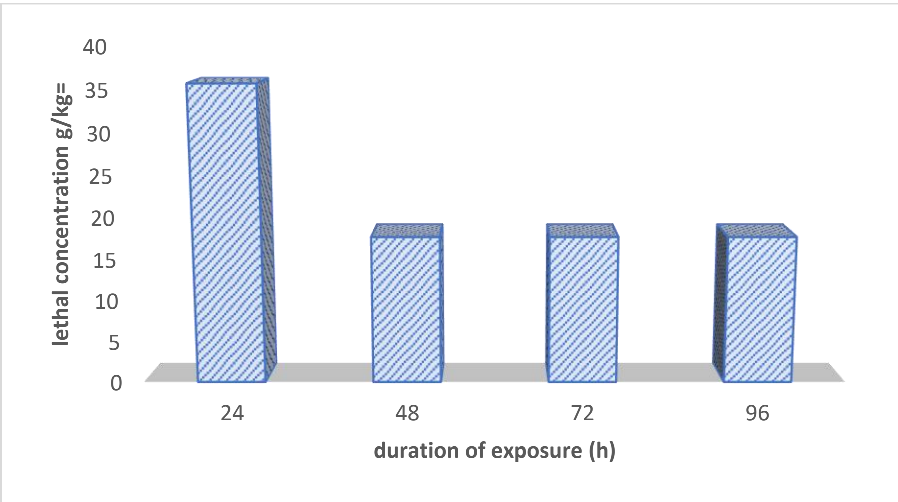

Figure 5: *LC_{50} of Neem powder as function of time*

DISCUSSION

The toxic effects of *A. indica*-based bio-insecticide extracts can effectively control corn weevils, while helping to control environmental pollution, and reducing consumer's diseases due to the use of synthetic insecticides. And this experiment was conducted by using the scientific usual methods presented above.

The yield of mechanical extraction of Neem oil is 7.06 %, which is much lower than the yields by solvents such as hexane, methanol and water which had 25.5 %, 12.5 % and 60 % respectively (Nyetam, 2022). However, the pure nature of this extract, which has not been subjected to any mixture of solvents, as well as the observed effectiveness, leaves no doubt about our results. The repellent toxicity tests of *A; indica* oil extracts made on *S. zea mays* showed repellent properties at all doses with an average of 62.5% corresponding to Mc Donald's class IV (Ndomo et *al.*, 2009), with a lethal or paralyzing effect that takes over the repellent properties at doses of 300 µl and 400 µl at the slightest contact with the oil. To kill 50 % of maize weevils (LC_{50}) within 24 hours, we need more Neem oil than within 48, 72, or 96 hours (decreasing quantity). The same thing tends to happen with the LC_{50} of Neem power only during the first 48 hours. The LC_{50} of the Neem powder after 72 or 96 hours of contact exposition tends to remain the same as that of 48 hours of exposition. These scientific data bring more precisions to Cameroonian or African users, who were using it informally until now.

Contact toxicity tests revealed insecticidal properties of the oil, ranging up to 90.72 % on the 4th day regardless of the dose. That mortality goes to 90.72 % from the first day for 400 µl (13,33µl/g), after applying mortality corrected by Abbott (1925), which takes into account the deaths observed in the control. However, since the oil extract is almost totally deadly from the first day, it can be assumed that insects that died of natural death would not have lasted more than two or three days in contact with the oil anyway. In that case the oil is 100 % lethal on the 4th day, for the smallest dose. From the first day the doses of 200 µl, 300 µl record a minimum mortality rate of 92 %, while at 400 µl the oil is 100% lethal on the 1st day. The powder extract, the control of which had no deaths, had a mortality of 93.33 % at doses of 1g and 5g, from the 2nd day, while the doses of 10 g and 15 g still only had 80 % and 86.66 % dead. From the 3rd day, the doses of 5 g, 10 g, and 15 g reach 100 %. According to Nyetam (2022), chemical insecticide call kill 50 % of the lab mousses within 3 hours of exposition by the respiratory way, while Neem seeds extracts have no negative effect by that same way. So, Neem seeds extracts sustainable and efficient solution.

On the other hand, according to Faye (2010), Debelmas and Hache tested the effect of the aqueous extract of neem on the central nervous system in 1976; No significant anticonvulsant, anticholinergic, analgesic, or sedative effects were observed. However, anti-stress effects, which would have been beneficial for our rats and mice, thus confirming the benefits that could be derived from a bio-insecticide based on Neem, compared to a synthetic insecticide, were observed, which reinforces our idea that Sinograin (chemical insecticide) has more negative impacts on health than *A. indica* (Nyetam, 2022). Our study comes in a context where Cameroonian and Africans farmers apart from some of them, do not know the use of Neem as an insecticide or pesticide, they do not have scientific data on the insecticidal virtues of Neem, do not master the dosages or dreads its bitterness. But illnesses and deaths (200,000 deaths/year), and the expenses caused by the synthetics pesticides, published by WHO and UNEP show us the dangers to which people are exposed (Anjarwalla, 2016). Our study is in line with the third and fifth goals of 2063 agenda, as well as the second sustainable development goal.

LIMITATIONS

The Neem seeds extracts have an embittered taste and a heavy smell that prevent from consuming directly the seeds that have been protected by our Bioinsecticide. Anyway, it may not cause bad effects on consumers' health such as chemical insecticides use to do. Another weakness is the unavailability of *Azadirachta indica*'s seeds.

This study will be completed with the study of others effect of Neem seeds extracts such as antifungal effects and also formulation of one or two Biopesticids. The bad smell and bitter taste will be removed by relevant phytopharmaceuticals and food technology methods.

CONCLUSION

At the end of this study, we have carried out repellent tests which showed the repellent properties of *Azadirachta indica*, making it possible to classify it in Mc Donald's class IV. The contact toxicity testing of *Azadirachta indica* oil has proven that it can achieve 100 % mortality, from day one at 400 µl (13.33 µl/g), and at all doses on day four for 30 g of corn. At doses of 200 µl and 300 µl, the mortality rate approaches 100 % from the first and second day. The mortality rate of the powder extract by contact is also close to 100 %, from 5 g. In 24 hours, the average lethal concentrations 50, are 94.62 µl (3.15 µl/g or 3.15 ml/kg) for oil and 1.07 g (35.66 g/kg) for *Azadirachta indica* powder.

The double problem of the destruction of maize stocks in storage and that of damage to the health of its consumers, due to synthetic insecticides, therefore finds a natural and reliable solution with the advantage of having a more positive than negative impact on the health. Many farmers in west Africa have starting using Neem seeds extracts to protect their crops. In this study, our producers will therefore, find the scientific proof and data allowing them to now use *Azadirachta indica* extracts with complete peace of mind. This study brings the food security and sustainable eating practice that have been lost with the use of synthetic insecticides that cause more than 200 000 deaths per year. It will equally contribute to avoid crops losses, improve earnings and fight against hunger.

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Conflicts of interest

The authors declare no conflict of interest.

List of authors and their contributions

NYETAM Benjamin is the main author of this part of the work which is from his Memories, he almost did all the work (study conception and design, experiments, data analysis, interpretation, manuscript writing, etc.) **Professor AMBANG Zachee** was the supervisor (study conception and design, data analysis, critical revision and approval**). Dr DONFAGSITELLI T. Nehemie** and **Dr TCHOKOUAHA YAMTHE Lauve** have also review it (data analysis, manuscript critical revision).

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LIST OF ABREVIATIONS

LC_{50}	:	Lethal mean Concentration killing 50 % of the population.
CRPMT	:	Center for Research on Medicinal Plants and Traditional Medicine.
DDT	:	Dichloro-Diphényl-Trichloréthane.
LD_{50}	:	Lethal mean Dose killing 50 % of the population.
FAO	:	Food and Agricultural Organisation of the United Nations.
IMPM	:	Institute of Medical Research and Medicinal Plants studies
IRAD	:	Institut de Recherches Agricoles pour le Développement (Institute of Agricultural research for development).
IRAM	:	Institut de Recherches et d'Applications des Méthodes de Développement (Institute for research and application of development methods).
IRDA	:	Institut de recherche et de développement en agroenvironnement(Institute for reseach and development in Agro-Environment).
MINADER	:	Ministry of Agriculture and rural development.
MINEPAT		Ministère de l'économie, de la planification et de l'aménagement du territoire
Mc	:	Corrected mortality.
MR(%)		Mortality rate
OECD		Organisation for economic cooperation and development.
SDG	:	Sustainable development goals.
PR	:	Percentage of repulsion.
SND30	:	National development Strategy 2020-2030.
UNEP		United Nations Environment Program.