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Terroir-Driven Efficacy : Harnessing the Chemogeographical Variation in *Vetiveria zizanioides* Essential Oils for Stage-Specific Management of the Cowpea Weevil (*Callosobruchus maculatus F*.)

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Abstract: *The global reliance on synthetic pesticides for preserving stored cowpea (Vigna unguiculata) seeds is jeopardized by rising insect resistance and food contamination, necessitating urgent exploration of safe, botanical alternatives. This study evaluates the biocidal and repellent potential of essential oils extracted from three geographically distinct Malagasy chemotypes of vetiver (Vetiveria zizanioides) Sambava, Fianarantsoa, and Tsiroamandidy against the devastating stored-product pest, Callosobruchus maculatus F. Chemical profiling revealed distinct compositions, with the Tsiroamandidy essential oil notably enriched in the sesquiterpenoids Khusimol (19.1%) and Zizanoic acid (15.1%). Bioassays demonstrated profound insecticidal effects proportional to concentration (10 to 80µl/l). The oils exhibited rapid fumigant toxicity, with all three chemotypes achieving 100% adult mortality at 60µl/l by 48 hours of exposure. Furthermore, the oils proved to be powerful reproductive inhibitors. Female fertility was completely suppressed at a concentration of 30µl/l, drastically reducing oviposition from the control average of 122 eggs. Most critically, the ovicidal activity was exceptional: the egg hatching rate and subsequent adult emergence (viability) were both completely inhibited (0%) even at the lowest tested dose of 10µl/l (control hatching rate : 82%). Beyond direct lethality, the essential oils demonstrated significant protection through repellency, classifying in the Highly Repellent (Class V) category at concentrations ≥60µl/l. These findings establish regional V. zizanioides essential oils as exceptionally potent, multi-action biopesticides, offering a sustainable and highly effective solution for cowpea preservation.*

Keywords: *Bioinsecticide, Callosobruchus maculatus, Cowpea, Essential oils*

I. Introduction

Food security remains a critical and escalating challenge for African nations, demanding increased agricultural productivity to meet rapid demographic growth. Successfully managing the biotic factors, particularly insect pest pressure on crops both in the field and in storage, is therefore essential (Akami et *al*., 2017)). Insect pests of stored foodstuffs cause catastrophic global losses, estimated by the FAO (2014) to reach up to 35% of food production. While synthetic chemical insecticides remain the dominant control method, their excessive use has resulted in severe environmental contamination and the

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emergence of resistant insect populations (Zhu et *al.*, 2022). This alarming reality necessitates the transition toward natural substances of botanical origin, which represent the most promising and ecologically sustainable alternative for pest control (Zhu et *al.*, 2022). Essential plant oils, recognized as less toxic, less polluting, and more economical (Et-tazy et *al.*, 2024), possess a wide array of bioactivities effective against agricultural and stored-product pests. Madagascar's favorable geographical location and rich vegetation provide an ideal resource for sourcing these natural biopesticides, a concept supported by traditional knowledge, where the efficacy of plant-based controls depends critically on the active ingredient (Akami et *al.*, 2017 ; Gueye, 2011 ; Et-tazy et *al.*, 2024).). Driven by these ecological and economic imperatives (Campolo et *al.*; Gueye, 2011 ; Et-tazy et *al.*, 2024), this study aims to rigorously assess the efficacy and durability of vetiver essential oils for the protection of cowpea seeds against the major stored-product pest, *Callosobruchus maculatus* F., in the Malagasy context.

II. Research Methods

2.1 Materials

a. Origin and morphological characterization of vetiver (*Vetiveria zizanioides*)

This study involved the comprehensive evaluation of vetiver essential oil sourced from three distinct geographical locations in Madagascar. Each sample was meticulously documented to ensure a precise correlation between its chemical profile and its origin.

1. Sample sourcing details

1) Vetiver 1 was collected in the Sava region, specifically from the Sambava district in the municipality of Farahalana, at the village of Benavony. The collection site is located 3 km south of Antohomaro village (14.2834°S, 50.1727°E). This specimen was harvested by Mbilo Jean Luc on October 31, 2018.
2) Vetiver 2 was obtained from the Haute Matsiatra region, in the Fianarantsoa district, municipality of Fianarantsoa I, at the Maromby Fokontany. The sample was collected 500 m north of the Maromby Trappist monastery (21.4373°S, 47.1057°E). This specimen was harvested by Jean Victor on November 13, 2018.
3) Vetiver 3 was sourced from the Bongolava region, specifically in the Tsiroamandidy district, Tsiroamandidy II municipality, at the Antaniditra Fokontany. This sample was collected 20 m east of the RN1b trunk road (18.7694°S, 46.0453°E). This specimen was harvested by Nambavelo Dyno on November 24, 2018.

2. Morphological and taxonomic characterization

All three analyzed specimens were taxonomically identified as belonging to the family Poaceae (Gramineae), within the genus *Vetiveria*, and designated as the species *Vetiveria zizanioides*.

This perennial, rhizomatous plant characteristically forms dense, vigorous clumps, supported by sturdy culms that typically reach impressive heights of 1.5 to 2 meters. The foliage is distinct, featuring thick, smooth, and glabrous sheaths that culminate in linear blades extending up to 1 meter in length. These blades are often noted for their bright yellow hue and possess a prominent keeled, overlapping base. The plant's reproductive structures are manifested as large, narrow, and oblong inflorescences, typically measuring between 25 and 30 cm.

The plant's most defining feature, critical for its essential oil production, is its subterranean architecture. The gnarled roots penetrate to significant depths, reaching 2 to 3 meters, while simultaneously forming a remarkably dense and highly branched network. Crucially, approximately 85% of the root mass is densely concentrated within the top 30 to 40 cm of the soil surface.

Locally, the species is recognized by the common Malagasy names "Le Verobe" or "Verofehana," and is known as "Vétiver" in the Réunion context. Herbarium voucher specimens are professionally preserved at the National Center for the Application of Pharmaceutical Research.

b. Cowpea (*Vigna unguiculata*) as the core medium

For the purposes of mass rearing within this investigation, cowpea seeds (*Vigna unguiculata*) were employed as the core medium.

The botanical classification of this vital legume underscores its position within the plant kingdom, tracing its lineage through a precise hierarchy: It is situated within the Plantae kingdom and the Dicotyledone class, falling under the Rosidae subclass and the Fabales order (sometimes classified as Rosales). The plant is a distinguished member of the Leguminosae family (alternatively Fabaceae), specifically the Papilionoidea subfamily. The cowpea, therefore, belongs to the Genus *Vigna*, culminating in the Species *unguiculata*, a systematic placement that speaks to its high nutritional value and biological suitability for large-scale propagation studies.

1. Nomenclature and sourcing

Historically, the plant was initially described by Linnaeus as *Dolichos unguiculatus* from a cultivated form originating in the West Indies, leading to its current binomial name, *Vigna unguiculata*.

Cowpea is known by a variety of names globally, including:

1) French : "Niébé," "Dolique de chine," and "Dolique manguette."
2) English : "Cowpea," "Blackeye bean," "Catjang," and "China pea."
3) Malagasy : "Voanemba" (Merina), "Vohemba" (Betsimisaraka), "Antsiroko" (Sakalava, Vezo), and "Lojy" (Antandroy).

For this study, healthy cowpea seeds were procured from the local Anosibe market in Antananarivo. The seeds were verified to be untreated with insecticides or other chemical compounds. They underwent rigorous cleaning (thoroughly washed, dried) and were subsequently stored at 4∘C prior to experimental use.

2. Biological model : The cowpea weevil (*Callosobruchus maculatus*)

This investigation utilized the cowpea weevil, *Callosobruchus maculatus* (Fabricius), as its primary biological model. This insect is an oligophagous species, notoriously recognized for its destructive capacity and its ability to transition seamlessly from field-to-storage infestations, with development often initiated before the host plants are even harvested.

Taxonomically, the weevil is classified within the Animalia kingdom and the Arthropoda phylum, belonging to the class Insecta and the order Coleoptera. Its systematic position continues through the sub-order Heterogastra. Although historically placed in the family Bruchidae, this family is now often considered invalid, with the species reclassified as a characteristic member of the Bruchinae subfamily within the family Chrysomelidae.

C. maculatus is a significant pest known for its post-embryonic development exclusively within the seeds of the Leguminosae (Fabaceae) family. Its classification culminates in the Genus *Callosobruchus* and the Species *C. maculatus*, with the cowpea (*Vigna unguiculata* L. Walp) serving as its principal host plant. This specific taxonomic placement highlights its specialized parasitic relationship and biological suitability for the study of seed-infesting pests.

3. Mass rearing conditions

The intensive mass rearing of the bruchid population was meticulously conducted over an eleven-month period, spanning from May 2018 to March 2019, at the laboratory of the National Center for the Application of Pharmaceutical Research.

To ensure a stable and sufficient insect population throughout the entire experimental duration, the cultures were maintained under strictly controlled environmental parameters: a constant temperature of 27°C (±1°C is often implied but 27°C is acceptable here) and a relative humidity of 75%. These specific, controlled parameters were established to optimize the growth and reproduction cycle of *Callosobruchus maculatus*.

2.2 Methods

a. Preparation and steam distillation of vetiver root

The preparatory steps preceding the distillation process are paramount to the successful extraction and ultimate quality of the desired essential oil. These crucial pre-distillation operations including washing, drying, and grinding must be carefully selected based on the specific extraction technique (such as hydro- or steam distillation) and the physical nature of the raw material (e.g., roots, bark, leaves, or seeds).

In the specific case of vetiver root, the material typically retains significant amounts of residue, such as sludge or earth, immediately following harvest. It is imperative that these extraneous contaminants be thoroughly removed, as their presence would otherwise compromise the purity and desired physicochemical properties of the final essential oil product. The efficient removal of this soil and/or sludge can be achieved through two primary methods: blasting or intensive washing.

b. Parameters of steam distillation

The essential oil was extracted using a controlled steam distillation method. The operation employed a stainless steel still with a considerable working capacity of 1500 liters. The process was meticulously monitored and maintained according to the following established parameters:

Parameter	Value
Distillation Type	Steam Distillation
Still Capacity	1500 liters
Operating Pressure	2.5 Bar
Distillation Time	22 hours
Hydrosol Flow Rate	110 liters per hour (l/h)
Hydrosol Temperature	34°C to 38°C

The prolonged distillation time of 22 hours is critical for ensuring the exhaustive recovery of the heavier, less volatile compounds characteristic of high-quality vetiver essential oil.

c. Biocide test

An experimental laboratory study was carried out in May 2018 at the “Centre National d'Application de Recherches Pharmaceutiques” (National Centre for the Application of Pharmaceutical Research) and the “Centre National de Recherche sur l'Environnement” (National Centre for Environmental Research), Antananarivo, Madagascar.

1. Evaluation of the efficacy of essential oils on *Callosobruchus maculatus* by inhalation

The inhalation effect of essential oils was studied on *Callosobruchus maculatus* adults using the method described by Papachristos and Stamopoulos (2002).

In the glass jars (1000ml capacity), cotton wools were attached by a thread to the center of the lids. Doses of essential oils: 0µl, 10µl, 30µl, 60µl, 80µl corresponding to calculated concentrations of 0, 10, 30, 60, 80µl/l volume of air, are injected into the cotton masses.

Five pairs of bruchids aged between 0 and 24 hours were quickly placed in the jars, which were immediately sealed with an adhesive strip. Three replicates were carried out for each dose and for the control.

Counts were taken after 24, 48, 72 and 96 hours in each jar. After correction, the observed mortalities are expressed using Abbott's mathematical expression (Abbott, 1925):

$$Pc = [(Po-Pt) /Ps] *100$$

With Pc: corrected mortality in %,
Pt: mortality observed in the control
Po: mortality observed in the trial.
Ps: live insects remain

2. Evaluation of the contact efficacy of essential oils on *Callosobruchus maculatus*

The effectiveness of the various contact treatments with vetiver essential oils on *Callosobruchus maculatus* was assessed by studying the longevity of adults, the fecundity and fertility of females and the viability of eggs using the essential oils.

1) Adult longevity

It is determined by counting, on a daily basis, the individuals who die after the tests have been launched for all doses, until all the individuals have died.

2) Female fertility

All the eggs laid on the seeds and boxes (hatched and unhatched) were counted using a binocular magnifying glass after 13 days of treatment.

3) Egg hatch rate

After counting the number of eggs laid, the hatching rate is calculated using the following formula:

$$\text{Hatching rate} = (\text{number of eggs hatched}/\text{number of eggs laid}) \times 100$$

Adults that begin to emerge from day 30 to day 40 are counted regularly and removed from the boxes as they emerge from the seeds.

The egg viability rate is calculated using the formula:

$$\text{Viability rate} = (\text{number of adults emerged}/\text{number of eggs laid}) \times 100$$

3. Evaluation of the repellent effectiveness of essential oils on *Callosobruchus maculatus*

This test is used to calculate the percentage of repulsion of an essential oil towards bruchid in the preferential zone on filter paper described by Jilani and Saxena (1990). Discs of filter paper 11 cm in diameter are cut into two equal parts; one half is treated with essential oil plus acetone and the other is treated with acetone only.

Doses of 0µl, 10µl, 30µl, 60µl, 80µl for the essential oils are diluted in 0.5 ml of acetone respectively, so that the oil is evenly distributed on the filter. The two half-discs of filter paper are air-dried and the disc is reconstituted and placed in a Petri dish.

Five pairs of bruchids aged between 0 and 24 hours were placed on filter paper in the middle of Petri dishes and four replicates were carried out for each dose and for each oil. After half an hour's treatment under laboratory conditions, the bruchids were counted on half-discs.

The percentage repulsion (PR) is calculated according to the formula used by Nerio (2009) as follows:

$$PR (\%) = [(Nac - Nh) / (Nac + Nh)] \times 100$$

With:

Nac: Number of bruches present on the half-disk treated with acetone

Nh: Number of bruchids present on the half-disk treated with the oil solution

The average percentage repellency for each oil is calculated and assigned to one of the different repellency classes ranging from 0 to V (McDonald, 1970**)**, which are presented below:

Table 1. Repulsion percentage according to the ranking of **Mc Donald et *al*.** (**1970**)

Classes	**Repulsion intervals**	**Properties**
Class 0	PR≤ 0.1%	Very low repellency
Class I	1%<PR≤ 20%	Weakly repellent
Class II	20%<PR≤ 40%	Moderately repellent
Class III	40%<PR≤ 60%	Moderately repellent
Class IV	60%<PR≤ 80%	Repellent
Class V	80%<PR≤ 100%	Highly repellent

d. Data collection

Enumeration is carried out according to the following timescales: after 30 minutes for the repulsion test, between 24 and 96 hours for the inhalation test, and from 1 to 40 days for the contact test. Each dose and the control are repeated four times.

e. Statistical analysis

The results obtained were subjected to an analysis of variance (ANOVA) to determine the significant differences between the treatments followed by the Bonferronni test and Dunnet's t-test with a probability level of $P<0.05$ using IBM SPSS Statistics 26 software.

III. Results and Discussion

3.1 Physicochemical characteristics of Vetiver essential oils

The composition of vetiver essential oils varies considerably depending on the composition of the soil and the extraction method. The unique physico-chemical properties of each oil influence its aromatic profile, its quality and its potential applications in various industries. To analyse these differences, we carried out a series of tests on three of our vetiver essential oils, the main characteristics of which are summarised in Table 2.

Table 2. Physico-chemical results for our vetiver oils

Physico-chemical constants	SAVA sample	TSIROAMANDIDY Sample	FIANARANTSOA sample
Density at 20°C	1.0227	1.032	1.021
Refractive index	1.5253	1.5214	1.5211
Specific rotation	+ 35°	+32°	+33°
Acid index	30.5	21.87	27.8
Ester index	14.5	8.415	5.7
Aspect	Thick liquid	Thick liquid	Thick liquid
Colour	Yellow, brown	Yellow, brown	Yellow, brown
Odour	Woody, pleasant	Woody, pleasant	Woody, pleasant

The analysis of the three liquid essential oil samples, sourced from Sava, Tsiroamandidy, and Fianarantsoa, revealed distinct physicochemical constants. All samples shared common organoleptic properties: they presented as thick, yellow-brown liquids and possessed a characteristically pleasant, woody aroma that was noted for its high persistence.

Quantitatively, the samples exhibited variations across key metrics :

- Density : The Tsiroamandidy sample recorded the highest density (1.032), whereas the Fianarantsoa sample presented the lowest value (1.021).
- Refractive Index : The refractive index values were relatively uniform across the three chemotypes, with the Sava sample showing the marginally highest reading (1.5253).
- Specific Rotation : The highest specific rotation was observed in the Sava oil (+35°), while the Tsiroamandidy oil recorded the lowest value (+32°).
- Acid and Ester Indices : Significant variations were also noted in the functional group indices. The Sava oil displayed the highest acid index (30.5), contrasting with the Tsiroamandidy oil, which registered the lowest ester index (8.415).

These differences in physicochemical parameters are crucial, as they reflect the distinct molecular compositions (chemotypes) influenced by the geographical and edaphic conditions of each sourcing region.

3.2 Analytical methodology for vetiver essential oil

The chemical composition of the three distinct vetiver essential oil samples sourced from Sava, Tsiroamandidy, and Fianarantsoa was rigorously determined via Gas Chromatography (GC).

a. Operating conditions

The analytical procedures were executed at the IMRA laboratory, employing precise, standardized operating conditions to ensure the accuracy and comparability of the results.

The analysis was performed using a TRACE 1300 Gas Chromatograph equipped with an AI 1310 automatic injector. Chromatographic separation was achieved utilizing a UB-WAX column (30m×0.32mm×0.5µm). The temperature programming for the oven was set to ramp from 50°C to 250°C at a controlled rate of 5°C/min.

The compounds were detected by a Flame Ionization Detector (FID), using Hydrogen as the carrier gas, maintained at a constant pressure of 0.50 bar. Sample introduction utilized the split mode with a ratio of 51:50. Finally, quantification was achieved through the area percentage method, with an established integration threshold set at 0.03%.
The following table summarises the results of the analysis:

Table 3. Chemical constituents by gas chromatography

Constituents	Concentration HE Sambava (%)	Concentration HE Tsiroamandidy (%)	Concentration HE Fianarantsoa (%)
β - vetivone	1.8	2.4	1.2
nootkatone	0.3	1.1	1.1
Khusimol	0.4	19.1	9.0
α - vetivone	11.0	11.5	2.4
Zizanoic acid	10 5	15.1	9.6

Essential oils from Sambava, Tsiroamandidy and Fianarantsoa have distinct chemical profiles. Tsiroamandidy is characterised by a high concentration of khusimol (19.1%) and zizanoic acid (15.1%), which may give it more pronounced aromatic and therapeutic properties. Sambava, with moderate concentrations of α-vetivone (11.0%) and zizanoic acid (10.5%), has a more balanced profile but lower levels of nootkatone (0.3%) and khusimol (0.4%). Fianarantsoa, although rich in khusimol (9.0%), is significantly lower in α-vetivone (2.4%), which sets it apart from the other oils.

The chromatographic spectra of the three essential oils studied are shown in the figures below.

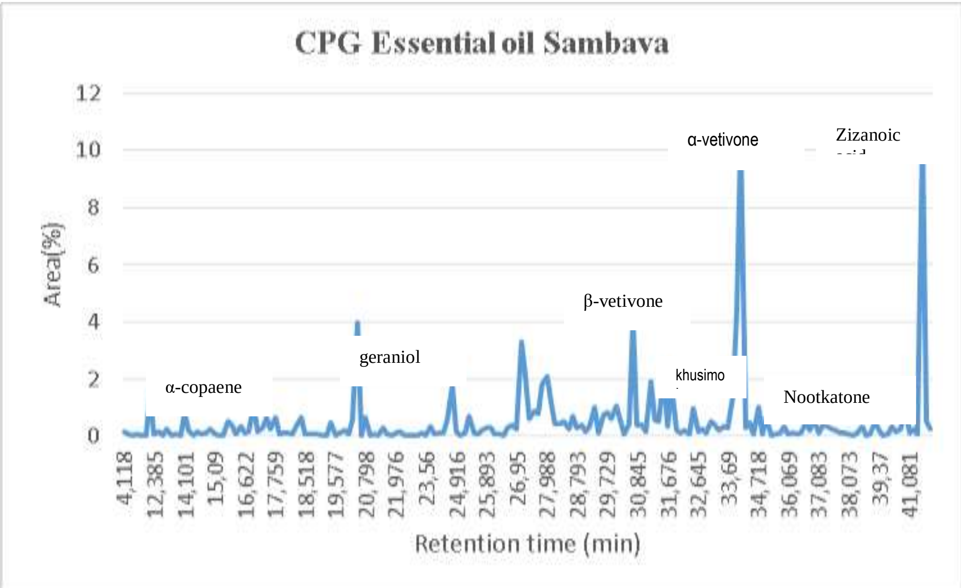

Figure 1. Chemical profile of Sambava Vetiver (*Vetiveria zizanioides*) essential oil

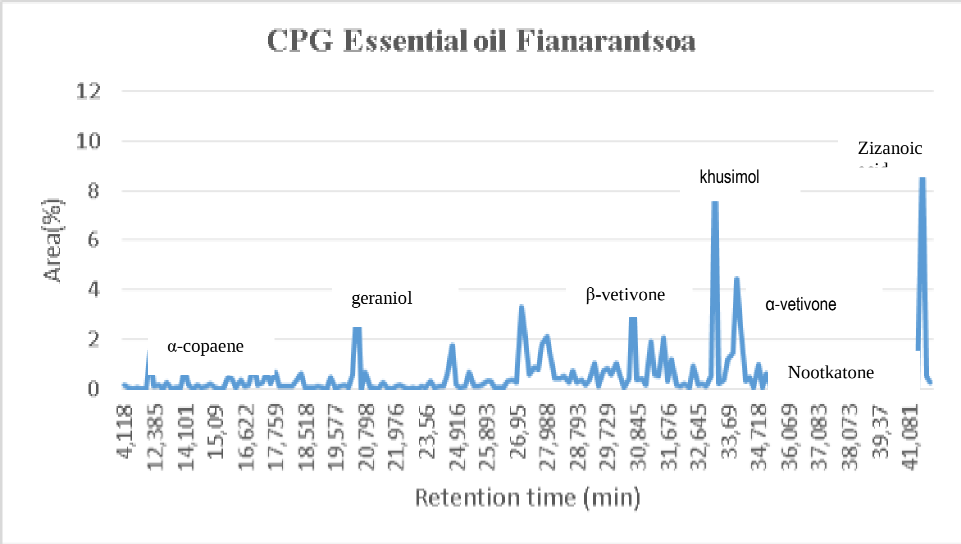

Figure 2. Chemical profile of Fianarantsoa Vetiver (*Vetiveria zizanioides*) essential oil

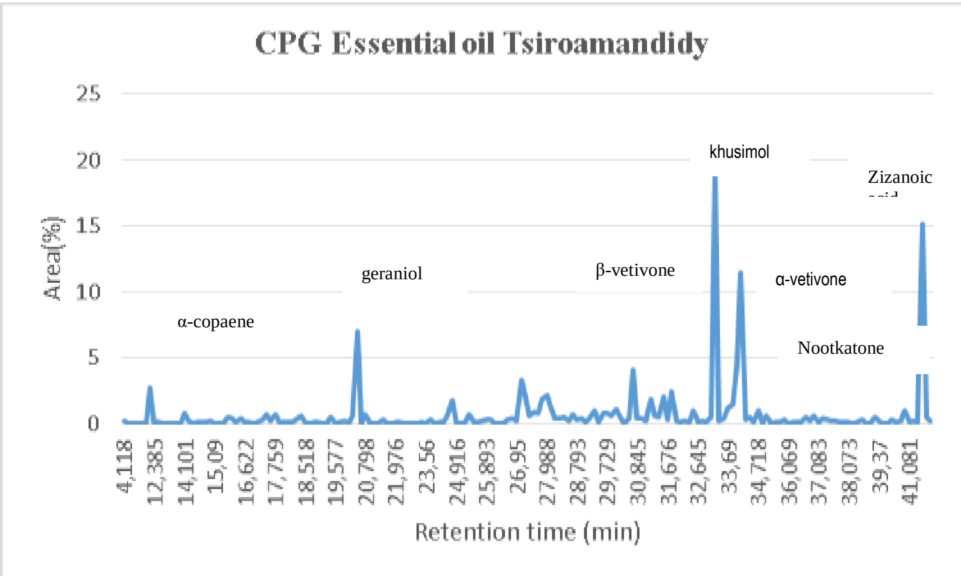

Figure 3. Chemical profile of Tsiroanomandidy Vetiver (*Vetiveria zizanioides*) essential oil

The chromatographic spectra of the three *vétiveria zizanoides* essential oils from Sambava, Tsiroamandidy, and Fianarantsoa show both similarities and differences in their chemical compositions. All samples contain β-vetivone, α-vetivone, khusimol, nootkatone, and zizanoic acid, indicating a similar qualitative profile. However, their relative proportions vary significantly among the three origins.

The oil from Tsiroamandidy is particularly rich in khusimol (19.1%) and zizanoic acid (15.1%), suggesting a higher oxygenated sesquiterpene content. Sambava oil is characterized by a high α-vetivone (11.0%) and zizanoic acid (10.5%) content, while Fianarantsoa oil presents generally lower concentrations of all major compounds, especially α-vetivone (2.4%) and β-vetivone (1.2%).

These differences may be attributed to environmental factors, soil composition and climatic variations between the three regions, which influence the biosynthesis of sesquiterpenes in vetiver roots.

3.3 The molecular eloquence of Vetiver : An ecological shield for cowpea

The Malagasy vetiver, sourced from distinct terroirs (Sambava, Fianarantsoa, and Tsiroamandidy), does not yield a uniform product, but a chemotypic palette where the concentration of oxygenated sesquiterpenoids (notably Khusimol and Zizanoic Acid) dictates the potency of the defense against the stored-product pest, the cowpea weevil (*Callosobruchus maculatus*).

Table 4. Chemotypic signature : The influence of terroir

Origin Site	Dominant chemotypic characteristic	Principal Oxygenated Sesquiterpenoids
Tsiroamandidy	Richest Chemotype	Khusimol (19.1%) and Zizanoic Acid (15.1%)
Sambava	Moderate and balanced profile	α-Vetivone (11.0%) and Zizanoic Acid (10.5%)
Fianarantsoa	Intermediate concentration profile	Khusimol (9.0%) and Zizanoic Acid (9.6%)

The Tsiroamandidy chemotype, enriched in these compounds, demonstrates the superiority of a dense molecular architecture for preserving seed heritage.

a. Evaluation of the efficacy of essential oils by inhalation on *Callosobruchus maculatus*

The efficacy of essential oils against *Callosobruchus maculatus* on cowpea seeds was assessed through inhalation tests, with the results summarised in Table 5. The experiment involved essential oils sourced from three different regions in Madagascar. Each essential oil's effectiveness was measured across different concentrations and time intervals.

This table presents the percentage mortality of cowpea weevils after exposure to Vetiver essential oil vapors from different geographical regions, measured over a 96-hour period.

Table 5. Effect of essential oil inhalation on cowpea weevil (*Callosobruchus maculatus*) mortality on seeds (%).

Dose (µl)	**Treatment**	**24h**	**48h**	**72h**	**96h**
Control	Indicator (Air/Solvent)	0	0	2	2
10	Essential oil (Sambava)	28	56	100	100
10	Essential oil (Fianarantsoa)	35	70	100	100
10	Essential oil (Tsiroamandidy)	50	90	100	100
30	Essential oil (Sambava)	33	67	100	100
30	Essential oil (Fianarantsoa)	55	75	100	100
30	Essential oil (Tsiroamandidy)	55	90	100	100
60	Essential oil (Sambava)	39	100	100	100
60	Essential oil (Fianarantsoa)	55	100	100	100
60	Essential oil (Tsiroamandidy)	60	100	100	100
80	Essential oil (Sambava)	61	100	100	100
80	Essential oil (Fianarantsoa)	65	100	100	100
80	Essential oil (Tsiroamandidy)	80	100	100	100

This table indicates the impact of essential oils from three different regions of Madagascar on the mortality of *Callosobruchus maculatus* through inhalation. The results show that all the essential oils tested showed biological activity. Tsiroanandidy essential oil was the most active, generally achieving 100% efficacy at lower concentrations and for shorter periods than the other oils. Increasing the concentration generally leads to an increase in efficacy. Essential oils from Sambava and Fianarantsoa have similar activity profiles. They are less active than that of Tsiroanandidy but show good efficacy.

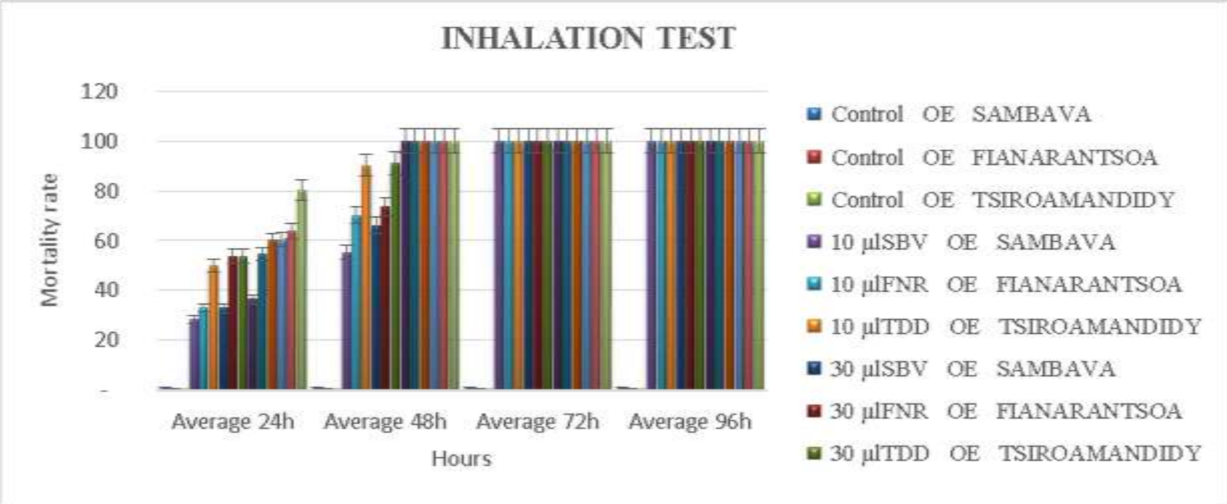

Figure 4. Evaluation of the efficacy of essential oils on *Callosobruchus maculatus* by inhalation

The three essential oils showed significant efficacy against *Callosobruchus maculatus*, and the insect mortality rate increased with the dose of oil used. A dose of more than 60 µl/l of air resulted in total insect mortality from the second day of exposure. On average, a dose of 30 µl/l of air caused 50% mortality on the first day. In addition, total mortality was observed on the third day for all doses tested, with the exception of the control.

Compound-activity relationship: Effective insecticidal activity via inhalation results from the sufficient volatility of sesquiterpenoids. These lipophilic molecules rapidly penetrate the insect's tracheal system. Their action is fundamentally neurotoxic, disrupting nerve impulse transmission, often by modulating receptors or octopaminergic channels. The high concentration of Khusimol (up to 19.1% in Tsiroamandidy) is strongly correlated with this rapid neurotoxicity. (Abdelgaleil and *al.*, ; 2024 ; Leite et *al*., 2023 ; Karabörklü & Ayvaz, 2023)

b. Effect of essential oils on the longevity of *Callosobruchus maculatus* by contact

The effect of vetiver essential oils on the longevity of *Callosobruchus maculatus* was assessed through direct contact exposure, with results summarised in Table 6. Mortality rates were recorded at intervals of 24, 48, 72, and 96 hours to measure the effectiveness of various oil doses across different collection sites.

Table 6. Effect of essential oils on the longevity of *Callosobruchus maculatus* by contact

Dose (µl)	**Treatment**	**24h**	**48h**	**72h**	**96h**
Control	Indicator (Air/Solvent)	2	1	2	1
10	Essential oil (Sambava)	50	71	100	100
10	Essential oil (Fianarantsoa)	44	76	100	100
10	Essential oil (Tsiroamandidy)	67	88	100	100
30	Essential oil (Sambava)	33	65	100	100
30	Essential oil (Fianarantsoa)	61	71	100	100
30	Essential oil (Tsiroamandidy)	56	88	100	100
60	Essential oil (Sambava)	61	100	100	100
60	Essential oil (Fianarantsoa)	61	100	100	100

60	Essential oil (Tsiroamandidy)	61	100	100	100
80	Essential oil (Sambava)	67	100	100	100
80	Essential oil (Fianarantsoa)	67	100	100	100
80	Essential oil (Tsiroamandidy)	83	100	100	100

This table shows the effects of different doses of essential oil on a percentage response indicator of mortality, measured at 24, 48, 72 and 96 hours. The controls, without essential oil, showed very weak responses, with percentages between 1 and 2% at all the times measured. On the other hand, the application of essential oil, whatever the dose and location, led to a progressive and significant increase in mortality rates, reaching 100% from 72 hours for all conditions. At 24 hours, responses varied according to dose and collection site, with higher percentages observed for oils from Tsiroamandidy. Increasing the dose of essential oil from 10 µl to 80 µl generally shows an increase in efficacy, although some samples (such as those of 60 µl and 80 µl) quickly reach the maximum of 100% after 48h. Finally, Tsiroamandidy essential oils are the most effective, particularly at higher doses.

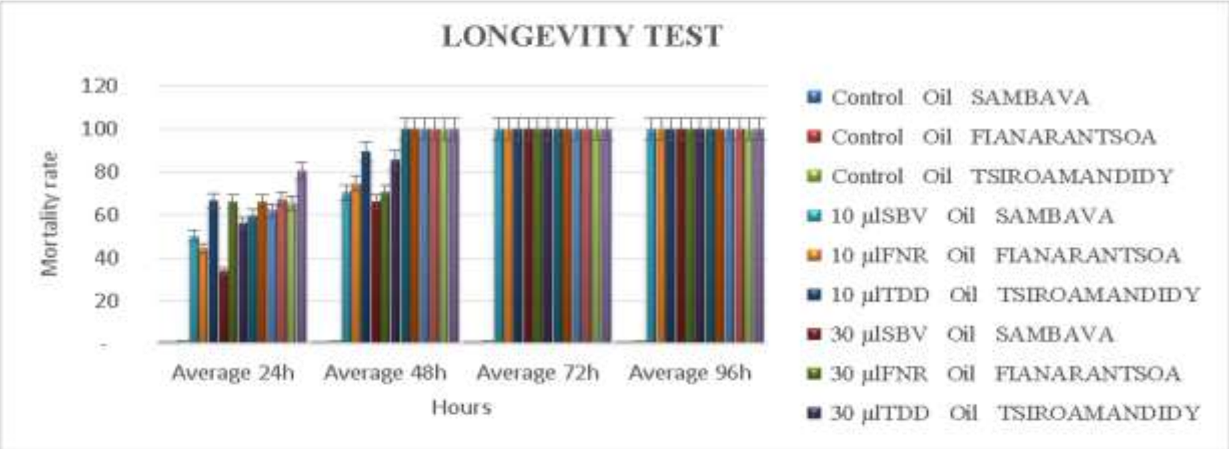

Figure 5. Effect of essential oils on the longevity of *Callosobruchus maculatus* by contact

By counting the number of dead individuals per day after the start of the tests for all doses, we observed that the three essential oils significantly reduced the longevity of *Callosobruchus maculatus* adults, results that are almost identical to those obtained in the inhalation tests. Of the doses tested, those of 60 µl/l and above resulted in total insect mortality from the second day of exposure for each oil. In addition, all doses, with the exception of the control, resulted in total insect mortality from the third day of exposure, while the control showed an average mortality of only 2%.

The one-way analysis of variance revealed highly significant differences (P=0) according to the doses applied, indicating the existence of distinct homogenous groups depending on the doses used.

Compound-activity relationship: Contact activity is directly linked to the high lipophilicity of the heavier sesquiterpenoids (Khusimol, Zizanoic Acid). Applied to the cowpea surface, these compounds adhere to the weevil's cuticle, dissolving its protective waxy lipid layer. This causes severe desiccation and cellular imbalance, leading to drastic reduction in adult lifespan (Isman 2023 ; Isman, 2020).

c. Effect of essential oils on the contact fertility of *Callosobruchus maculatus* females

The effect of vetiver essential oils on the fertility of female *Callosobruchus maculatus* was investigated through direct contact. This analysis aims to understand how different doses of essential oils influence reproductive outcomes in this pest.

The results are presented in histograms for enhanced clarity.

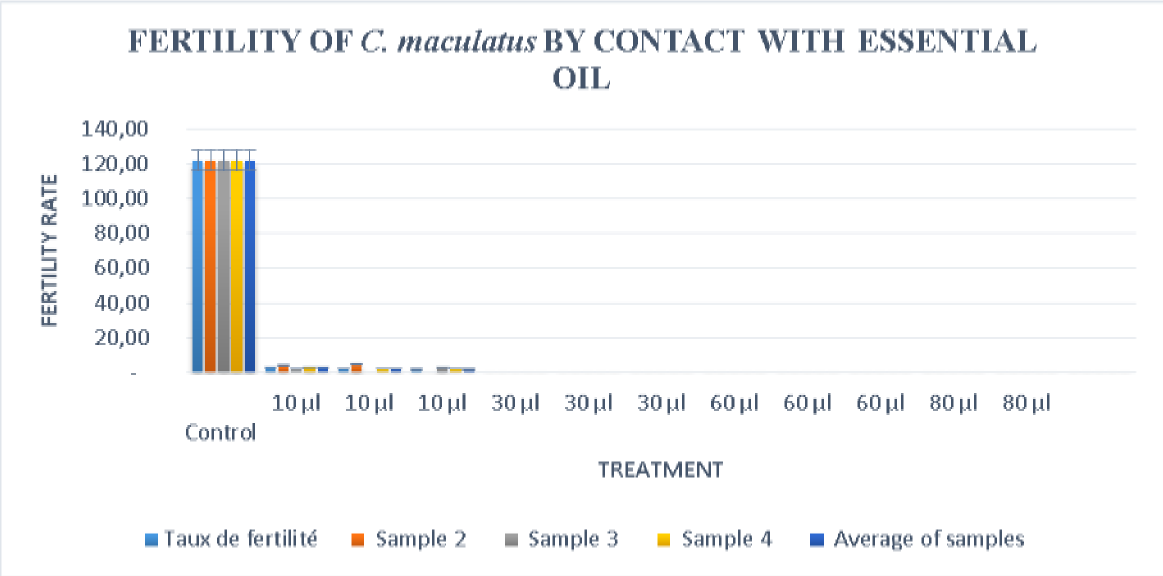

Figure 6. Effect of essential oils on the fertility of *Callosobruchus maculatus* females by contact

The total number of eggs laid, encompassing those deposited on both the seeds and the inner walls of the containers (regardless of hatching status), was meticulously quantified using a binocular magnifying glass 13 days after the initiation of treatment.

The data, summarized in the corresponding figure, establishes the control group (untreated) baseline at a constant average of 122 eggs across all three geographical localities. A marked dose-dependent inhibitory effect was observed even at the lowest concentration tested: at a dose of 10 µl/l, the average number of eggs laid sharply decreased to just 2.75 (Sambava), 2.25 (Fianarantsoa), and 1.75 (Tsiroamandidy). This massive reduction equates to an average fertility rate of only 3% across the treated groups at this concentration.

Crucially, the ovicidal activity demonstrated that fertility was completely inhibited starting from a dose of 30 µl/l. At this point, the weevils were effectively sterilized, recording zero viable offspring. These powerful inhibitory results were rigorously supported by Dunnett's t-test, which yielded a highly significant p-value of 0.000. This statistical outcome strongly confirms a profound inverse dependency between the applied essential oil dose and the resultant reduction in insect fertility when compared to the untreated control.

Compound-activity relationship: The nearly complete suppression of fertility and egg hatching suggests Insect Growth Regulator (IGR) activity (Assadpour et *al.*, 2024 ; Gupta et *al.*, 2023). The oxygenated sesquiterpenoids penetrate the eggshell (chorion) to inhibit embryonic cell division or act upon the female, disrupting hormonal pathways essential for oogenesis (yolk formation).

d. Effect of essential oils on the hatching of eggs laid by *Callosobruchus maculatus* on contact

This section investigates how vetiver essential oils affect the hatching of eggs laid by *Callosobruchus maculatus* through direct contact. The findings aim to demonstrate the influence of different essential oil doses on egg viability and the subsequent hatching process. The results are illustrated in figure 7.

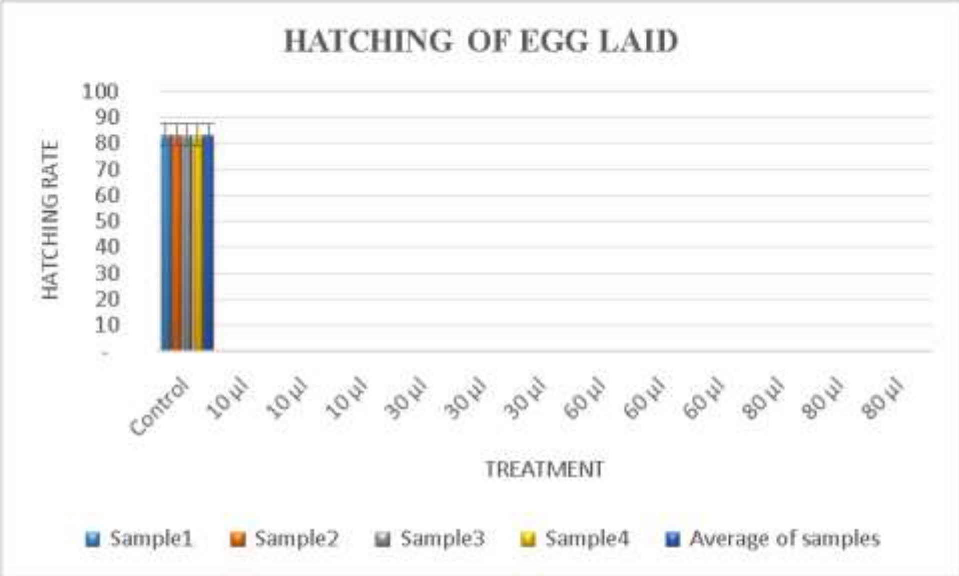

Figure 7. Effect of essential oils on the hatching of eggs laid by *Callosobruchus maculatus* as a result of contact.

The figure illustrates the sample averages for the control group and the various essential oil doses. The untreated controls across all localities yielded a consistent constant value of 83.33% (presumably the mean number of viable eggs or the overall hatching percentage in the control batches, though the former interpretation is more likely given the subsequent 82% figure). This homogeneity suggests that the experimental conditions did not inherently affect egg viability across the different geographical setups.

Following the counting of eggs laid, the control batches were observed to have an average hatching rate of 82%. However, for all samples treated with essential oil, the results were definitive: the hatching rate was completely inhibited (0%) starting from the lowest tested dose of 10 µl/l.

This profound ovicidal impact was further validated through statistical analysis. Analysis of variance (ANOVA) revealed highly significant differences (with a P-value of 0) depending on the dose of the essential oils, unequivocally confirming the marked inhibitory effect of these doses on egg hatching. In essence, the essential oil acts as a highly effective ovicide, preventing the successful post-embryonic development of the weevil.

This is the pinnacle of preventive efficiency : vetiver imposes a posterity lock. By annihilating egg hatching at minimal concentrations (as low as 10 µL/L), these molecules effectively sever the species' reproductive cycle at its root, ensuring the biological sterilization of the stored grain without relying on toxic residues. (Assadpour et *al.*, 2024 ; Gupta et *al.*, 2023)

e. Effect of essential oils on the viability of *Callosobruchus maculatus* hatching eggs (or emergence) by contact

This section assesses how vetiver essential oils influence the viability of *Callosobruchus maculatus* hatchlings through direct contact. The results are depicted in Figure 8, highlighting the relationship between essential oil doses and hatchling emergence.

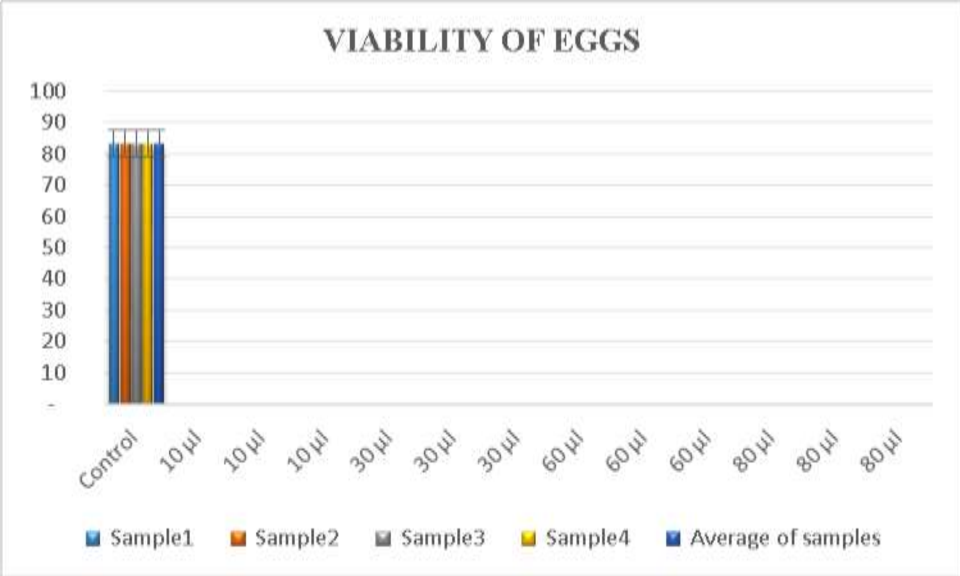

Figure 8. Effect of essential oils on the viability of *Callosobruchus maculatus* eggs hatched on contact

The figure illustrates the sample averages for adult emergence across controls and various essential oil doses. The control groups for all localities showed a uniform average value of 8.33 (representing the mean emergence rate or number of emerging adults), which suggests stability and homogeneity of results in the absence of treatment.

Adult weevils were observed to begin emergence from approximately the 30th day until the 40th day post-treatment. During this period, emerging adults were regularly counted and promptly removed from the Petri dishes as soon as they exited the seeds.

The results definitively established that egg viability was zero starting from the lowest tested dose of 10 µl/l for all three essential oils. Specifically, the emergence rate recorded for the 10 µl, 30 µl, 60 µl, and 80 µl doses was 0 for all samples and locations.

In stark contrast, the untreated control batch maintained a high overall hatching rate of 82%. Statistical analysis using Dunnett's test confirmed that the essential oil doses had a highly significant inhibitory effect on egg viability, with a P-value of 0 (indicating $P<0.001$). This result unequivocally demonstrates the profound and complete ovicidal effectiveness of the vetiver essential oils.

1. Repellent activity of Vetiver essential oils against *Callosobruchus maculatus*

This section rigorously examines the repellent effects of vetiver essential oils on the cowpea weevil, *Callosobruchus maculatus*. The findings demonstrating the dose-dependent response are meticulously summarized in Table 7, which details the percentage of repulsion achieved at various doses and across the different collection sites.

To assess relative efficacy, the average percentage repellency for each oil was calculated and subsequently categorized into one of the standardized repellency classes defined by McDonald et *al*. (1970). These classes, which range from Class 0 (Non-Repellent) to Class V (Highly Repellent), allow for a clear, standardized interpretation of the protective potential of the essential oils.

Table 7. Effects of vetiver essential oils on *Callosobruchus maculatus*

Dose (µl)	**Treatment**	**Replicate 1 (%)**	**Replicate 2 (%)**	**Replicate 3 (%)**	**Replicate 4 (%)**	**Mean Mortality Rate (%)**
Control	Negative control (Air)	0.000	0.000	0.000	0.000	0.000

10	Essential oil (Sambava)	42.857	42.857	42.857	42.857	42.860
10	Essential oil (Fianarantsoa)	25.000	42.857	42.857	42.857	38.390
10	Essential oil (Tsiroamandidy)	25.000	66.667	42.857	42.857	44.350
30	Essential oil (Sambava)	42.857	66.667	66.667	66.667	60.710
30	Essential oil (Fianarantsoa)	66.667	66.667	66.667	66.667	66.670
30	Essential oil (Tsiroamandidy)	66.667	66.667	66.667	66.667	66.670
60	Essential oil (Sambava)	100.000	66.667	100.000	100.000	91.670
60	Essential oil (Fianarantsoa)	66.667	100.000	100.000	100.000	91.670
60	Essential oil (Tsiroamandidy)	100.000	100.000	100.000	100.000	100.000
80	Essential oil (Sambava)	66.667	100.000	100.000	100.000	91.670
80	Essential oil (Fianarantsoa)	100.000	66.667	100.000	100.000	91.670
80	Essential oil (Tsiroamandidy)	100.000	100.000	100.000	100.000	100.000

Figure 9 shows the effects of vetiver essential oils on the repusivity of *Callosobruchus maculatus*.

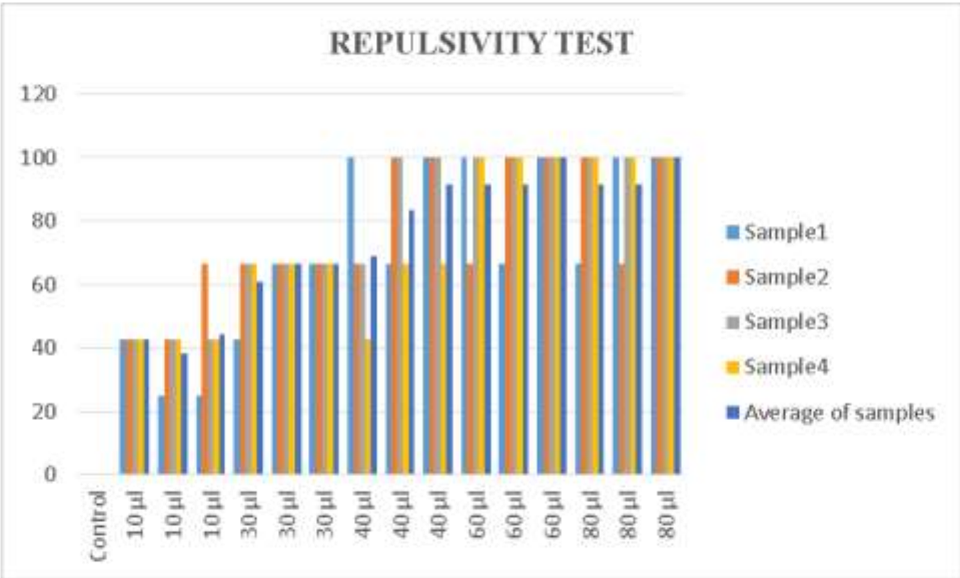

Figure 9. Effect of essential oils on the repellency of *Callosobruchus maculatus.*

After treating the bruchids for half an hour under laboratory conditions, the insects were counted on the half-discs. The results show that the repellency of the essential oils is proportional to the dose applied. At a dose greater than 60 µl/l, the oils are classified as highly repellent. At 30 µl/l, they are considered repellent, while at the lowest dose of 10 µl/l, they are moderately repellent.

Compound-activity relationship: The essential oils achieve a Highly Repellent status (Class V) due to the intense organoleptic properties of their sesquiterpenoid profile (Vetivones and Khusimol). This powerful olfactory shield masks the cowpea's attractive chemical signals, inducing an avoidance reaction by affecting the insect's antennal chemoreceptors. (Elbrense et *al*., 2021 ; Devi et *al*., 2020)

2. Effect of essential oils on cowpea seed weight loss

This section focuses on evaluating how essential oils affect the weight loss of cowpea seeds. The findings are illustrated in Figure 10, which highlights the correlation between the dosage of essential oils and the resulting seed weight loss.

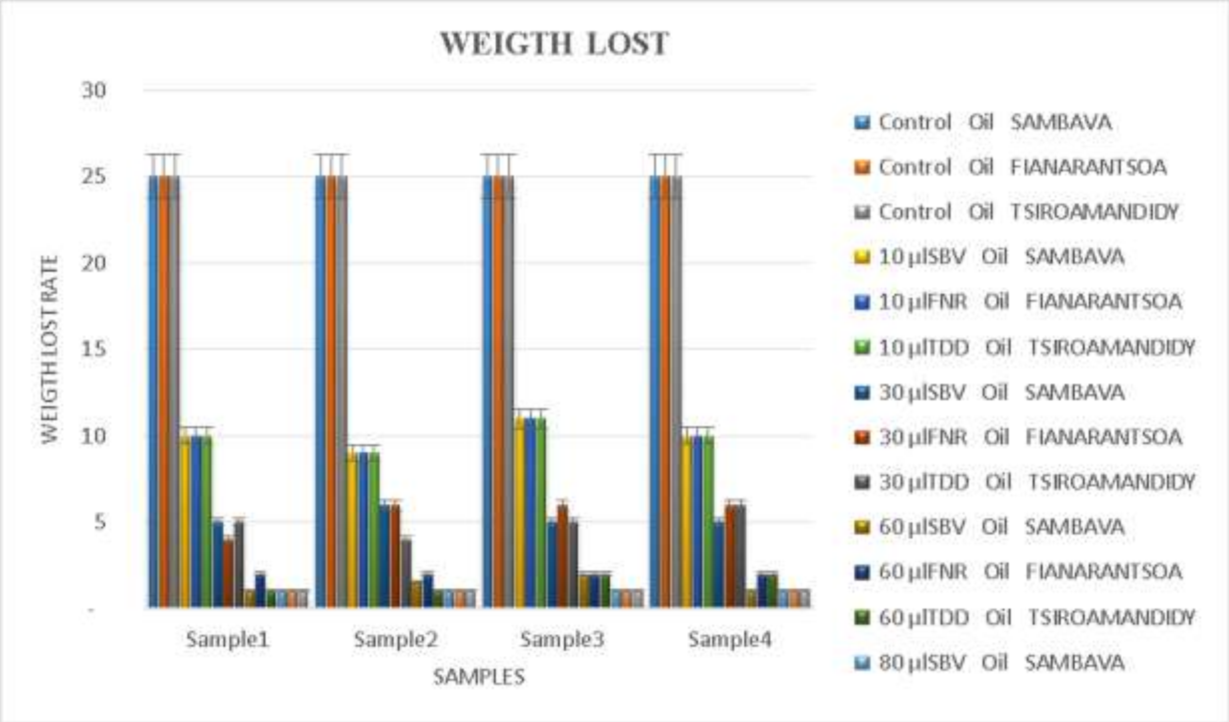

Figure 10. Effect of essential oils on cowpea seed weight loss

The weight loss sustained by the cowpea seeds following the emergence of the first adult generation was quantified by comparing the final weight of the treated batches against their initial pre-treatment weight using a precision balance.

The results clearly established that untreated control samples suffered a substantial mean weight loss of 25% across all localities due to weevil infestation.

In contrast, the application of essential oils dramatically curtailed this damage:

1) Low Dose Efficacy : The minimum dose of 10 µl/l (the lowest dose tested) halved the loss, reducing the average weight loss to a uniform 10% across all three geographical origins, indicating a consistent initial protective response.
2) Intermediate Doses : By increasing the dosage to 30 µl/l, the protective effect was significantly enhanced, driving the mean weight loss down to 5% (Sambava and Tsiroamandidy) and 6% (Fianarantsoa).
3) Maximum Protection : The loss was virtually eliminated at doses of 60 µl/l and above. At 60 µl/l, the average loss fell to 1% (Sambava) and 2% (Fianarantsoa and Tsiroamandidy). Finally, at the highest dose of 80 µl/l, the averages were uniformly reduced to 1% for all locations, demonstrating saturation of the protective effect at this minimal damage threshold.

These findings unequivocally confirm the high protective potential of vetiver essential oils, demonstrating their capacity to maintain the quantity and economic value of stored cowpea seeds.

Compound-activity relationship: Success in these two areas is the direct, integrated consequence of all biocidal and repellent activities. By sterilizing the eggs (ovicidal) and preventing larval development inside the seed, the oils stop the feeding cycle. The preservation of germinative capacity confirms that the oil concentration, while lethal to the weevil, exhibits no significant phytotoxicity to the plant embryo. (Bakkali et *al*., 2008)

3. Effect of essential oils on seed germination

To assess the impact of essential oil treatments on seed germination, 25 seeds were taken at random from each batch tested. These seeds were placed in Petri dishes containing cotton soaked in water. A control batch is made up of untreated (healthy) seeds, as well as seeds infested by bruchids (for the contact treatment control test). Figure 11 shows the effect of essential oils on seed germination.

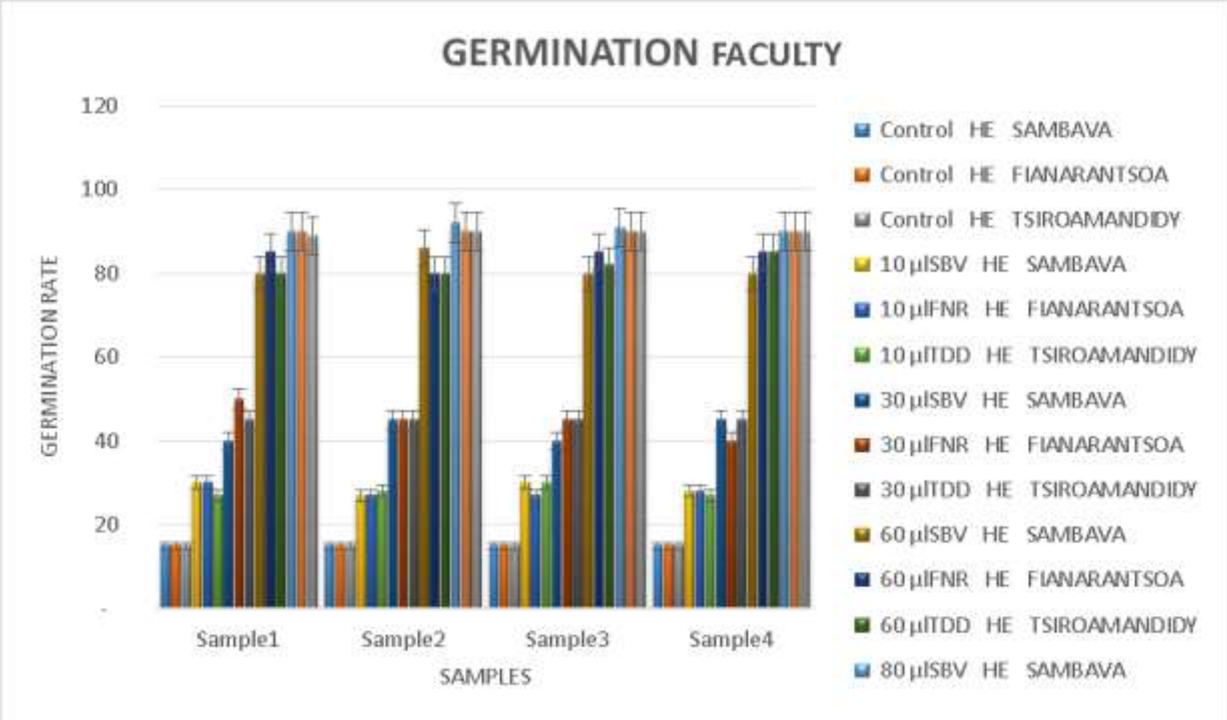

Figure 11. Effect of essential oils on the germinative capacity of seeds

This figure shows the effect of different doses of essential oils on the germinative capacity of the seeds as a percentage. The control samples have a constant average of 15% for each location, indicating a uniform base value. With a dose of 10 µl, the averages increase to 29% for Sambava, 28% for Fianarantsoa, and 28% for Tsiroamandidy, showing a positive response to the treatment. Moving up to 30 µl, the averages rise considerably, reaching 43% for Sambava, 45% for Fianarantsoa, and 45% for Tsiroamandidy, indicating an even greater effect. Doses of 60 µl produced very high averages, of 82% for Sambava, 84% for Fianarantsoa, and 82% for Tsiroamandidy, showing a significant maximum effect. Finally, at 80 µl, the averages stabilized at around 91% for Sambava, 90% for Fianarantsoa, and 90% for Tsiroamandidy, suggesting that the maximum effect was reached with this concentration.

After 4 days of experimentation, we counted the number of germinated seeds. The results show an inverse correlation with weight loss: as the treatment dose increases, the seeds are less damaged and the germination rate increases. This rate was 15% for the controls and reached an average of 85% for the dose higher than 60 µl.

IV. Conclusion

This investigation conclusively validates the potent, multifaceted bio-efficacy of Malagasy Vetiver essential oil (*Vetiveria zizanioides*) as a sustainable alternative to synthetic insecticides for cowpea preservation. The study's comparative approach across three regional chemotypes (Sambava, Fianarantsoa, Tsiroamandidy) confirmed that efficacy is dose-dependent and linked to the unique chemical profile of the oil.

The Tsiroamandidy chemotype, characterized by its high concentration of Khusimol (19.1%) and Zizanoic acid (15.1%), demonstrated the fastest action. Critically, all tested oils exhibited superior performance across all bioassays:

1. Lethal Toxicity : Complete adult mortality (100%) was achieved through fumigation at doses ≥60µl/l of air within just 48 hours. Total mortality via contact exposure was reached for all conditions at 72 hours.
2. Reproductive Inhibition : The essential oils proved to be highly effective chemosterilants.

Female fertility was completely inhibited starting at a dose of 30µl/l. Even more striking, the ovicidal effect was instantaneous, resulting in 0% egg hatching and 0% adult emergence from the seeds at the lowest tested concentration of 10µl/l.

1. Seed Protection : This biopesticidal action translated directly into tangible protection of the stored commodity. Seed weight loss, which reached 25% in untreated controls, was virtually eliminated (reduced to 1%) at doses ≥60µl/l. Consequently, the seed germination rate increased from a low of 15% in controls to an average of 85% at protective doses.
2. Repellency : Doses ≥60µl/l provided an excellent protective barrier, classifying the oils as Highly Repellent (Class V).

Through a mere aromatic breath, vetiver erects an intangible wall. These vapors, rich in sesquiterpenoids, act as volatile neuronal inhibitors, delivering a swift, curative strike to the pest. This method of control necessitates no direct contact with the stored reserves, thereby minimizing the environmental footprint of the treatment. The seed gains surface immunity. The molecular architecture of the sesquiterpenoids disrupts the insect's protective exoskeleton, causing physiological stress and premature mortality. This is a strategy of progressive, non-persistent control that limits the pest's window of nuisance and reproductive activity. Vetiver establishes an aromatic force field. Its deep, earthy scent, while pleasant to us, acts as an alarm signal for the weevil, preventing it from landing and contaminating the seeds. This is a non-damage strategy, where protection is guaranteed through olfactory deterrence, preventing harm without needing to dispatch the organism. By completely controlling the pests, these essential oils act as faithful guardians of agricultural biodiversity. They ensure that the seed, which is both our food and our promise of future harvest, retains its intrinsic vitality and full potential for perpetuating the cycle of life.

In synthesis, the multifaceted efficacy combining rapid adult mortality, total reproductive suppression, and strong repellency confirms that *V. zizanioides* essential oil is a powerful and viable botanical protectant. Its successful application offers a crucial step toward economically sound and ecologically sustainable management of stored cowpea seeds against *Callosobruchus maculatus* in Madagascar and similar tropical environments.

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