



Vetiver Root Extract as a Biopesticide Against *Callosobruchus maculatus* F., insect destructive of cowpea seeds (*Vigna unguiculata* L. Walp.) in stock

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Abstract: This investigation rigorously assessed the bioinsecticidal potential of methanolic root and leaf extracts derived from *Vetiveria zizanioides* sourced from three distinct Malagasy chemotypes (Sambava, Fianarantsoa, and Tsiroamandidy). The primary goal was to validate a sustainable, botanical alternative for preserving stored cowpea (*Vigna unguiculata*) against the destructive pest, *Callosobruchus maculatus*. The vetiver root extracts demonstrated potent, dose-dependent efficacy across all bioassays. Specifically, at concentrations of 60 µl/l or higher, the extracts induced complete adult mortality within 72 hours via contact, and nearly total mortality within 48 hours via fumigation. The Tsiroamandidy chemotype proved particularly efficacious, achieving total mortality by the second day at the maximum dose. Critically, the extracts exhibited profound ovicidal activity, resulting in zero egg hatchability even at the minimum concentration of 10 µl/l, completely suppressing reproduction. This decisive biological action translated into significant commodity protection: seed weight loss, which was 25% in untreated controls, was virtually eliminated at the 60 µl/l threshold, consequently preserving seed quality and germination viability. In sharp contrast, the leaf extracts showed severely limited efficacy, achieving a maximum mortality of only 40% even at the highest tested dose. These findings conclusively validate the vetiver root extract as a highly promising, natural biopesticide for the sustainable management of *C. maculatus*.

Keywords: Vetiver extract, *Callosobruchus maculatus*, cowpea, bioinsecticide.

I. Introduction

Addressing food security remains a critical global imperative, particularly among populations in African countries, where agricultural output must sustainably match rapid demographic growth. It is essential to enhance productivity by effectively managing the numerous edaphic, climatic, and biological factors that intensify pest pressures on crops, both in the field and during storage (Baoua et al., 2017). Pests that attack stored products, notably insects,

contribute to significant quality and quantity losses. Indeed, the FAO (2020) reports that post-harvest losses from pest-related damage impact up to 30% of global yields. Although chemical insecticides remain the predominant method for controlling these pests, their widespread and often uncontrolled use has resulted in both extensive food chain contamination and the rapid development of widespread pesticide resistance (**Kumar et al., 2017**).

Consequently, botanical alternatives, especially plant extracts, present an effective and ecologically sound approach to pest control, exhibiting potent bioactivity against a wide spectrum of agricultural and stored-product insects. Madagascar, with its unique and diverse flora, serves as a rich source for numerous aromatic plants that can be sustainably leveraged for natural insecticide production (**Togola et al., 2020**). The adoption of biopesticides, such as essential oils derived from these plants, holds significant potential for reducing environmental pollution and preserving the quality of vital crops like cowpea (*Vigna unguiculata*), which is highly susceptible to post-harvest infestation (**Hajam et al., 2022**). It is worth noting that in rural settings, small-scale farmers have long employed traditional methods, including the incorporation of plant extracts, ashes, and oils, to mitigate pest infestation in stored grains.

The efficacy of plant-based insecticides depends not merely on the volume of seeds to be protected, but critically on the specific active compounds present across different plant organs and, consequently, in the final extract (**Hajam et al., 2022**). From both ecological and economic perspectives, the development of effective, sustainable biopesticide alternatives to synthetic pesticides is paramount for robust pest control in both agricultural and storage contexts (**Baoua et al., 2017**). This study was thus initiated to rigorously assess the effectiveness and durability of vetiver-based essential oil extracts in the preservation of cowpea seeds against *Callosobruchus maculatus* infestations in Madagascar.

II. Research Methods

2.1 Plant materials

a. Botanical source : Vetiver (*Vetiveria zizanioides*)

This study utilized three distinct chemotypes of vetiver (*Vetiveria zizanioides*), all collected in late 2018 from geographically diverse regions in Madagascar. Each specimen was meticulously documented to establish a precise correlation between its geographical origin and its resultant phytochemical profile. All herbarium voucher specimens are professionally preserved at the National Centre for the Application of Pharmaceutical Research.

Sample sourcing details

Specimen ID	Region / District	Collector	Collection Date	Coordinates
VESBV18	Sava / Sambava	Mbilo Jean Luc	October 31, 2018	14.2834°S, 50.1727°E (3 km S of Antohomaro)
VEFNR18	Haute Matsiatra Fianarantsoa	/ Jean Victor	November 13, 2018	21.4373°S, 47.1057°E (500 m N of Maromby Monastery)

Taxonomic and morphological characterization

All collected specimens are taxonomically classified under the species *Vetiveria zizanioides*, belonging to the genus *Vetiveria* and the family Poaceae (formerly Gramineae).

This perennial rhizomatous species forms dense, vigorous, and long-lasting clumps, supported by tall, sturdy culms that typically reach heights of 1.5 to 2 meters. The foliage consists of yellow-green, smooth-edged leaves up to 1 meter in length. Reproductive structures manifest as slender, elongated inflorescences, typically measuring between 25 and 30 cm (sometimes reaching 40 cm), supported by delicate racemes 5 to 10 cm long.

The plant's most defining characteristic is its extensive root system, which descends to significant depths of 2 to 3 meters. Crucially, approximately 85% of the total dense, fibrous root mass is concentrated within the upper 30 to 40 cm of the soil surface. The common vernacular names for the species are "Le Verobe" or "Verofehana" (Malagasy) and "Vétiver" (Ansari et al, 2020).

b. Cowpea seeds (*Vigna unguiculata*)

The cowpea plant, utilized as the core medium for insect mass rearing, was initially described by Linnaeus as *Dolichos unguiculatus* (based on a West Indies variety), leading to its current binomial name, *Vigna unguiculata* (Dugie et al., 2009).

Taxonomic hierarchy

Level	Classification
Kingdom	Plantae
Class	Dicotyledonae
Order	Fabales
Family	Leguminosae (Fabaceae)
Genus	<i>Vigna</i>
Species	<i>unguiculata</i>

Nomenclature and sourcing

The cowpea is known by various vernacular names, including Niébé (French) and Black-eyed Bean (English). In Malagasy, it is known as Voanemba (Merina), Vohemba (Betsimisaraka), Antsiroko (Sakalava, Vezo), and Lojy (Antandroy). (Togola et al., 2019)

For the purpose of mass rearing, healthy seeds were sourced from the Anosibe market in Antananarivo. The seeds were verified to be untreated with any insecticide or chemical compound. They were subsequently thoroughly washed, air-dried, and stored at a constant temperature of 4°C until required for experimental use.

2.2 Animal material: The cowpea weevil (*Callosobruchus maculatus*)

Taxonomic classification and ecology

The primary biological model for this study was the cowpea weevil, *Callosobruchus maculatus* (Fabricius). This insect is an oligophagous beetle belonging to the order Coleoptera. It is specifically adapted to perform its post-embryonic development exclusively within legume seeds (Family Fabaceae), with the cowpea (*Vigna unguiculata* L. Walp.) serving as its principal host plant.

Taxonomically, the weevil is classified as follows:

Hierarchy Level	Classification
Kingdom	Animalia
Phylum	Arthropoda
Class	Insecta
Order	Coleoptera
Family	Chrysomelidae
Subfamily	Bruchinae
Species	<i>Callosobruchus maculatus</i>

It is important to note that the *Bruchidae* family is now commonly recognized as the Bruchinae subfamily within the larger Chrysomelidae family. A key ecological characteristic of this pest is its ability to initiate infestation in the field prior to harvest, subsequently setting up massive losses in storage (Ekoja et al., 2022).

Mass rearing conditions

For the purpose of the investigation, a stable and sufficient population of *Callosobruchus maculatus* was intensively reared over an eleven-month period, spanning from May 2018 to March 2019. This mass rearing was conducted under controlled laboratory conditions at the Centre National d'Application des Recherches Pharmaceutiques. The cultures were consistently maintained at a constant temperature of 27°C and a relative humidity of 75% to optimize the growth and reproduction cycle throughout the experimental duration. (Hajam et al., 2022)

2.3 Methods

a. Preparation of methanolic extracts

The preliminary steps for the alcoholic extraction were crucial to ensure the quality of the final plant extract. Prior to the process, the vetiver leaves and roots (the raw materials) were subjected to thorough washing, air-drying, and fine grinding. The extraction utilized a 24-hour alcoholic maceration technique with methanol as the solvent. Maceration was conducted at a controlled temperature range of 34°C to 38°C. Following the maceration period, the solvent was removed via vacuum evaporation using a rotavapor to yield the final, concentrated crude extract. The total extraction time, including maceration and evaporation, lasted approximately 26 hours (Sarker et al., 2017).

b. Biocidal activity testing

An experimental laboratory study was carried out in May 2018 at both the Centre National d'Application des Recherches Pharmaceutiques (CNARP) and the Centre National de Recherche sur l'Environnement (CNRE) in Antananarivo, Madagascar. The biocidal activity of the extracts was evaluated against adult *Callosobruchus maculatus* across three primary mechanisms: fumigation, contact, and repellency (Regnault-Roger et al., 2012).

Fumigant toxicity (Inhalation assay)

The effect of the extract by inhalation was evaluated on adult *C. maculatus* using a modified method based on Papachristos and Stamopoulos (2002). Assays were conducted in 750 ml glass jars. Doses of 0 µl, 10 µl, 30 µl, 60 µl, and 80 µl were injected into a cotton wool wick tied to the centre of the jar lids, corresponding to concentrations of 0, 10, 30, 60, and 80 µl/l of air, respectively. Five pairs of bruchids (aged 0 to 24 hours) were rapidly introduced into each jar,

which was then sealed hermetically with adhesive tape. Each dose, including the untreated control, was tested in triplicate.

Mortality observations were recorded after 24,48,72, and 96 hours. Observed mortality rates were corrected using Finney's formula (**Finney, 1971**) :

$$P_c = \frac{P_o - P_t}{P_s} \times 100$$

Where P_c is the corrected mortality percentage, P_o is the mortality observed in the treated trial, P_t is the mortality observed in the control, and P_s represents the number of live insects remaining in the control.

Contact efficacy and reproductive assays

The efficacy of the contact treatments was assessed by measuring the impact of the extracts on four distinct biological parameters:

- Adult longevity : Determined by daily counting and removal of dead individuals in each dose until 100% mortality was reached.
- Female fecundity (fertility) : All laid eggs (hatched and unhatched) on both seeds and container walls were counted using a binocular magnifying glass after 13 days of treatment.
- Egg hatching rate : Calculated after counting the total number of laid eggs using the formula (**Tapondjou, et al., 2002**) :

$$\text{Hatching Rate (\%)} = \frac{\text{Number of eggs hatched}}{\text{Number of eggs laid}} \times 100$$

- Egg viability rate (emergence): Adults emerging between the 30th and 40th days were counted and immediately removed. The viability rate (**Hajam et al., 2022**) was determined by:

$$\text{Viability Rate (\%)} = \frac{\text{Number of adults emerged}}{\text{Number of eggs laid}} \times 100$$

Repellent activity

The repellent effectiveness was evaluated using a preference test on filter paper, adapted from the method described by **Jilani and Saxena (1990)**. An 11 cm diameter filter paper disk was divided into two halves. One half was treated with a mixture of the extract solution and acetone, while the other (control) half was treated with acetone only. The same extract doses (0 μ l, 10 μ l, 30 μ l, 60 μ l, and 80 μ l) were prepared by diluting them in 0.5 ml of acetone. The halves were air-dried, reassembled, and placed in a Petri dish.

Five pairs of bruchids (0 to 24 hours old) were placed in the center, and the number of insects present on each half-disc was counted after 30 minutes. Each dose and each oil was tested in quadruplicate. The percentage repulsion (PR) was calculated using the formula described by **Nerio et al. (2009)** :

$$PR (\%) = \frac{(N_{ac} - N_h)}{(N_{ac} + N_h)} \times 100$$

Where N_{ac} is the number of bruchids on the acetone-only (control) half-disk and N_h is the

number of bruchids on the extract-treated half-disk. The calculated average PR for each oil was then assigned to one of the repellency classes (0 to V) defined by **McDonald et al. (1970)**. 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:

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

c. Data analysis and statistical methods

Data quantification was performed at specific time intervals: 30 minutes for the repulsion test, between 24 and 96 hours for the fumigant toxicity test, and from 1 to 40 days for the contact efficacy assays. Each dose, including the control, was replicated four times.

Results were subjected to Analysis of Variance (ANOVA) to identify significant differences between the treatment groups. This was followed by post-hoc analyses using Bonferroni and Dunnett's t-tests. Statistical analysis was conducted using IBM SPSS Statistics 26 software, with the level of significance set at $P < 0.05$.

III. Results and Discussion

3.1 Qualitative phytochemical screening of ethanolic extracts of vetiver

Alcoholic extracts of leaves and roots of *Vetiveria zizanioides*, collected from three distinct regions (Sambava, Tsiroamandidy, Fianarantsoa), were subjected to a phytochemical screening to detect the presence and distribution of major secondary metabolites.

Table 1. Qualitative phytochemical screening

Phytochemical groups	Leaf Sambava	Leaf Tsiroanomandidy	Leaf Fianarantsoa	Root Sambava	Root Tsiroanomandidy	Root Fianarantsoa
Alkaloids	+	+	+	+	+	+
Flavonoids	++	++	+++	+	+	+
Tannins	++	++	++	+	+	+
Saponins	++	+	++	—	—	—
Sterols	+	+	+	++	+++	++
Total phenols	+	+	+	+	+	+
Sesquiterpenic ketone	+	+	+	++	+++	++
Sesquiterpenic alcohol	+	+	+	++	+++	++
Sesquiterpenic acid	+	+	+	++	+++	++
Coumarins	+	+	+	—	—	—
Anthocyanins	+	+	+	—	—	—
Total terpenes	+	+	+	++	+++	++

— : absent ; + : weak ; ++ : moderate ; +++ : strong presence

The qualitative phytochemical screening revealed a clear distinction between leaf and root extracts.

Leaf extracts from the three regions showed high levels of flavonoids, tannins, phenolic compounds, and saponins, with slightly higher intensities observed in Fianarantsoa samples. In contrast, root extracts exhibited a strong presence of oxygenated sesquiterpenes as well as triterpenes and sterols, especially in samples from Tsiroanomandidy. These variations indicate that both geographical origin and plant organ play a significant role in the biosynthesis and accumulation of secondary metabolites in Malagasy vetiver.

3.2 Evaluation of fumigant toxicity against *Callosobruchus maculatus*

The insecticidal potential of the three methanolic vetiver extracts (root and leaf) was initially assessed through fumigant toxicity against adult *C. maculatus* beetles. The results (Table 1) clearly demonstrate a dose-dependent effect, with root extracts (RT) exhibiting significantly higher efficacy than leaf extracts (LF) across all tested concentrations and exposure times.

Table 2. Effect by inhalation of vetiver extracts against *Callosobruchus maculatus* on Cowpea seeds. (RT: root, LF: leaf)

Dose (µl)	Type	Cultivation site	24h (%)	48h (%)	72h (%)	96h (%)
Control	-	-	1	1	2	2
10	RT	Sambava	30	75	94	94
10	RT	Fianarantsoa	25	60	94	94
10	RT	Tsiroamandidy	30	70	94	94
10	LF	Sambava	10	25	12	12
10	LF	Fianarantsoa	10	13	13	13
10	LF	Tsiroamandidy	10	13	13	13
30	RT	Sambava	40	80	85	85
30	RT	Fianarantsoa	25	60	90	90
30	RT	Tsiroamandidy	35	65	90	90
30	LF	Sambava	10	13	30	30
30	LF	Fianarantsoa	10	19	35	35

30	LF	Tsiroamandidy	-	19	35	35
60	RT	Sambava	45	90	100	100
60	RT	Fianarantsoa	45	65	100	100
60	RT	Tsiroamandidy	50	85	100	100
60	LF	Sambava	15	25	40	40
60	LF	Fianarantsoa	10	25	40	40
60	LF	Tsiroamandidy	-	13	30	30
80	RT	Sambava	65	90	95	95
80	RT	Fianarantsoa	45	90	100	100
80	RT	Tsiroamandidy	75	100	100	100
80	LF	Sambava	20	13	40	40
80	LF	Fianarantsoa	10	13	40	40
80	LF	Tsiroamandidy	15	13	30	30

(RT: root, LF: leaf)

For the root extracts, a high level of toxicity was rapidly achieved. At the 60 µl/l air volume dose, 100% adult mortality was registered by the 72-hour mark for all three chemotypes (Sambava, Fianarantsoa, and Tsiroamandidy). Notably, the 80 µl dose of the Tsiroamandidy root extract demonstrated exceptional virulence, achieving complete mortality within just 48 hours of exposure. Even at the lowest concentration tested (10 µl), root extracts maintained strong efficacy, causing an average of 70% mortality within 48 hours and stabilizing at 94% after 72 hours. In contrast, leaf extracts (LF) proved largely ineffective, reaching a maximum mean mortality of only 40% at the highest dose applied. This disparity confirms the superior concentration of active fumigant compounds within the vetiver root tissue.

3.3 Effect of vetiver extracts on *C. maculatus* longevity by contact

The influence of the methanolic extracts on the adult lifespan of *C. maculatus* was investigated via direct contact toxicity (Table 2). Consistent with the fumigant results, the **root extracts** drastically reduced insect longevity in a dose-dependent manner, while the leaf extracts showed minimal impact.

Table 3. Effect of vetiver extracts on the longevity of *Callosobruchus maculatus* by contact

Dose (µl)	Type	Place of cultivation	24h (%)	48h (%)	72h (%)	96h (%)
Control			-	-	-	-
10	RT	Sambava	22	71	69	75
10	RT	Fianarantsoa	17	53	69	81
10	RT	Tsiroamandidy	22	59	88	88
10	LF	Sambava	-	12	12	12
10	LF	Fianarantsoa	-	13	13	13
10	LF	Tsiroamandidy	-	13	13	13
30	RT	Sambava	33	76	81	81
30	RT	Fianarantsoa	17	53	88	88
30	RT	Tsiroamandidy	28	65	85	94
30	LF	Sambava	-	13	13	13
30	LF	Fianarantsoa	-	19	19	19
30	LF	Tsiroamandidy	-	19	19	19

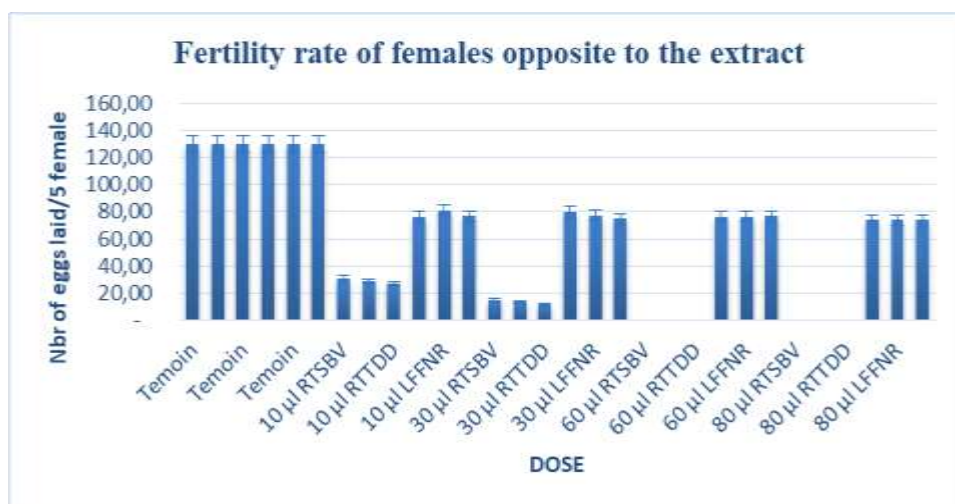
60	RT	Sambava	39	88	100	100
60	RT	Fianarantsoa	39	59	94	100
60	RT	Tsiroamandidy	44	82	100	100
60	LF	Sambava	6	25	25	25
60	LF	Fianarantsoa	-	25	25	25
60	LF	Tsiroamandidy	-	13	13	13
80	RT	Sambava	61	88	100	100
80	RT	Fianarantsoa	39	88	100	100
80	RT	Tsiroamandidy	72	100	100	100
80	LF	Sambava	11	13	25	25
80	LF	Fianarantsoa	-	13	25	25
80	LF	Tsiroamandidy	6	13	13	13

(RT: root, LF: leaf)

Root extracts at 60 μ l and 80 μ l concentrations resulted in 100% mortality by the 72-hour post-treatment period for nearly all root chemotypes. The Tsiroamandidy root extract was again the most potent, achieving full mortality at the 80 μ l dose in just 48 hours, underscoring its rapid contact neurotoxic action. Conversely, the 10 μ l dose caused an average 80% mortality by the third day, a strong result that remained unchanged thereafter. Statistical analysis via one-way Analysis of Variance (ANOVA) confirmed highly significant differences based on the doses ($P=0$), positioning the Tsiroamandidy root extract as the most effective in reducing the longevity of *C. maculatus*. The leaf extracts, however, consistently failed to produce meaningful mortality, peaking at only 25% at the highest doses tested.

3.4 Effect of extracts on the fertility of *Callosobruchus maculatus* females by contact

To assess the potential of the extracts to disrupt the reproductive cycle, female fertility was measured (Graph 1).



(RT: root ; LF: leaf ; SBV : Sambava ; TDD : Tsiroanomandidy ; FNR : Fianarantsoa)

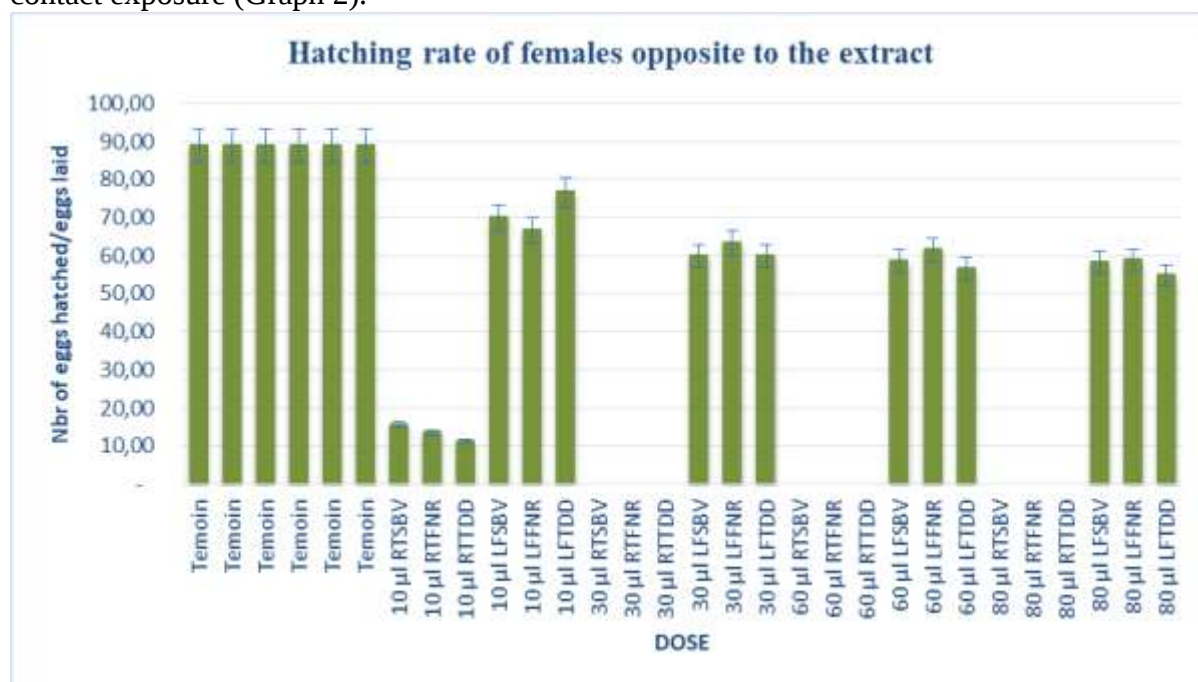
Graph 1. Effect of vetiver extracts on the fertility of *Callosobruchus maculatus* females by contact

The results clearly indicate that the fertility rate is inversely proportional to the dose of root extract applied. At concentrations of 60 μ l and above, fertility was entirely suppressed across all three root chemotypes. This profound anti-fertility effect was statistically

confirmed by the Dunnett test ($P=0.000$), indicating a strong and significant dependence on the extract dose. In stark contrast, the hypothesis test for leaf extracts showed non-significant results, reinforcing their failure to substantially impact the reproductive capacity of the female bruchids.

3.5 Effect of vetiver extracts on the hatching of eggs laid by *Callosobruchus maculatus* by contact

The ovicidal potential was examined by measuring the rate of egg hatching following contact exposure (Graph 2).



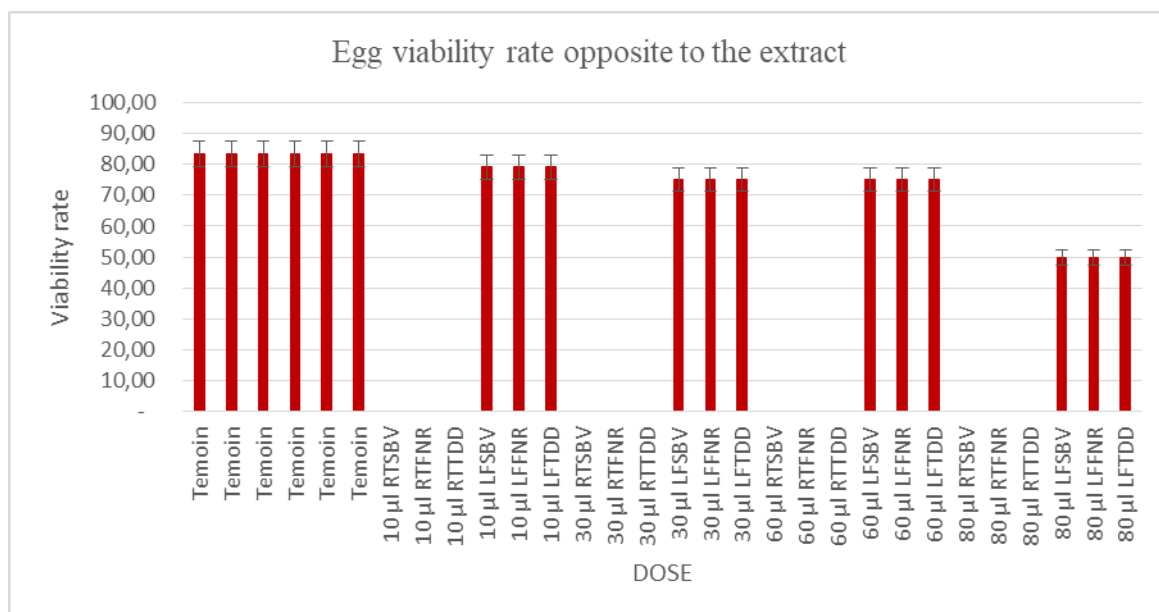
(RT: root ; LF: leaf ; SBV : Sambava ; TDD : Tsiroanomandidy ; FNR : Fianarantsoa)

Graph 2. Effect of vetiver extracts on the hatching of eggs laid by *Callosobruchus maculatus* by contact

The control sample exhibited a high hatching rate of 82%. Astonishingly, all three **root extracts** demonstrated complete ovicidal activity, resulting in a zero hatching rate even at the minimum tested dose of 10 µl. This instantaneous and absolute inhibition of embryonic development highlights the root extracts' exceptional ability to prevent the next generation of pests. The ANOVA confirmed a highly significant dose factor for root extracts ($P=0$). In sharp contrast, the leaf extracts showed non-significant differences by ANOVA, with the hatching rate remaining relatively high (an average of 50% even at the maximum 80 µl dose).

3.6 Effect of extracts on egg viability (emergence) by contact

The viability rate (successful emergence of adults from laid eggs) provides the ultimate measure of control over the pest's life cycle (Graph 3).



(RT: root ; LF: leaf ; SBV : Sambava ; TDD : Tsiroanomandidy ; FNR : Fianarantsoa)

Graph 3. Effect of vetiver extracts on the viability of eggs hatched by *Callosobruchus maculatus* by contact

As expected, the results closely mirror the ovicidal findings. Dunnett's test (Montgomery, 2017) confirmed a highly significant impact of the root extract doses on egg viability ($P=1.136 \times 10^{-11}$). While the control viability reached 82%, the high efficacy of the root extracts effectively eliminated emergence. Conversely, leaf extracts, even at the maximum dose of 80 µl, allowed for an average viability rate of 50%, further underlining their poor protective value compared to the root extracts.

3.7 Repellent activity

The repellency of the methanolic extracts against *C. maculatus* was evaluated using a preferential zone test (Table 3). The repellency rate proved to be directly proportional to the dose of the extract applied.

Table 4. Effect of vetiver extracts on *Callosobruchus maculatus* by repulsion

Dose (µl)	Type	Cultivation site	Sample1	Sample 2	Sample 3	Sample 4	Average of samples
Control			0.000	0.000	0.000	0.000	0.00
10	RT	Sambava	25.000	25.000	42.857	42.857	33.93
10	RT	Fianarantsoa	25.000	25.000	25.000	25.000	25.00
10	RT	Tsiroamandidy	25.000	25.000	25.000	25.000	25.00
10	LF	Sambava	25.000	25.000	25.000	25.000	25.00
10	LF	Fianarantsoa	11.111	42.857	25.000	25.000	25.99
10	LF	Tsiroamandidy	25.000	25.000	25.000	25.000	25.00
30	RT	Sambava	42.857	66.667	42.857	42.857	48.81
30	RT	Fianarantsoa	66.667	42.857	66.667	66.667	60.71
30	RT	Tsiroamandidy	66.667	42.857	66.667	66.667	60.71
30	LF	Sambava	42.857	42.857	42.857	42.857	42.86
30	LF	Fianarantsoa	25.000	25.000	42.857	42.857	33.93
30	LF	Tsiroamandidy	42.857	42.857	25.000	25.000	33.93
40	RT	Sambava	66.667	42.857	42.857	66.667	54.76

40	RT	Fianarantsoa	66.667	42.857	42.857	66.667	54.76
40	RT	Tsiroamandidy	66.667	42.857	42.857	66.667	54.76
40	LF	Sambava	25.000	25.000	25.000	42.857	29.46
40	LF	Fianarantsoa	25.000	25.000	25.000	42.857	29.46
40	LF	Tsiroamandidy	42.857	42.857	42.857	25.000	38.39
60	RT	Sambava	66.667	42.857	66.667	66.667	60.71
60	RT	Fianarantsoa	66.667	42.857	66.667	66.667	60.71
60	RT	Tsiroamandidy	66.667	42.857	66.667	66.667	60.71
60	LF	Sambava	25.000	25.000	42.857	42.857	33.93
60	LF	Fianarantsoa	25.000	66.667	42.857	42.857	44.35
60	LF	Tsiroamandidy	42.857	42.857	66.667	66.667	54.76
80	RT	Sambava	66.667	42.857	66.667	66.667	60.71
80	RT	Fianarantsoa	66.667	42.857	66.667	66.667	60.71
80	RT	Tsiroamandidy	66.667	42.857	66.667	66.667	60.71
80	LF	Sambava	25.000	25.000	42.857	25.000	29.46
80	LF	Fianarantsoa	25.000	25.000	66.667	42.857	39.88
80	LF	Tsiroamandidy	42.857	42.857	42.857	25.000	38.39

(RT: root, LF: leaf)

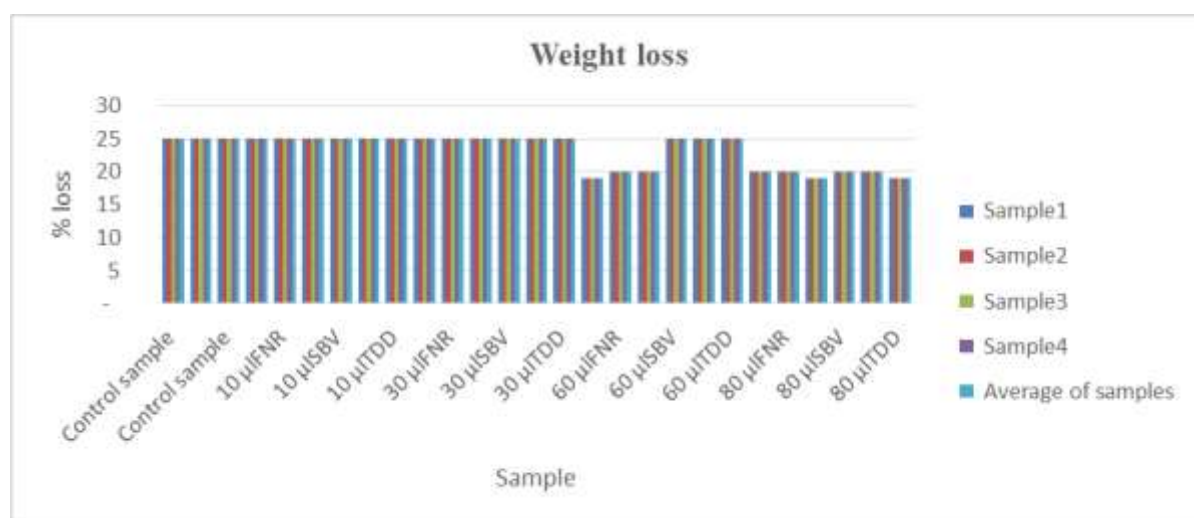
Root extracts displayed effective repellent properties:

- At the lowest dose (10 μ l), they were classified as Moderately Repellent (Class II/III).
- At 30 μ l, they transitioned into the Repellent category (Class III/IV, with two chemotypes reaching 60.71%).
- At doses of 60 μ l and 80 μ l, the root extracts maintained strong repellency (averaging 60.71% for all three chemotypes), classifying them as Repellent (Class IV) according to the McDonald *et al.* (1970) scale.

In contrast, leaf extracts demonstrated consistently lower and less significant repellency, remaining classified as Moderately Repellent (Class III) even at the highest concentration tested.

3.8 Effect of vetiver extracts on cowpea seed weight loss

Seed weight loss is a critical indicator of the degree of damage caused by *C. maculatus* infestation (Graph 4).



(RT: root ; LF: leaf ; SBV : Sambava ; TDD : Tsiroanomandidy ; FNR : Fianarantsoa)

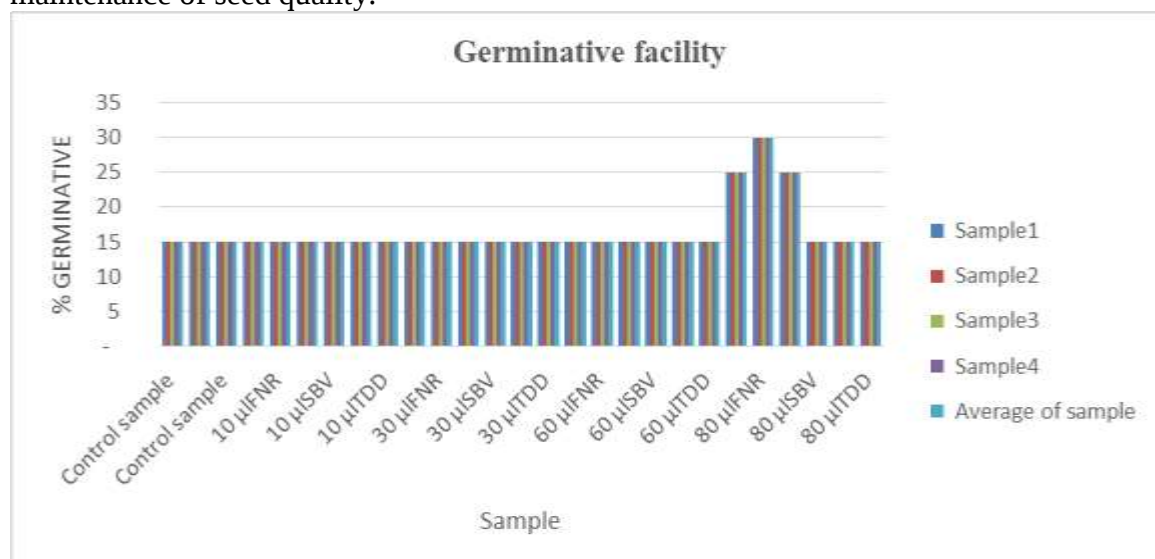
Graph 4. Effect of vetiver extracts on cowpea seed weight losses

The analysis aimed to assess the commodity protection afforded by the extracts. The results, when interpreted in the context of the biocidal findings, show a strong protective effect, particularly at high concentrations.

At low doses (10 μ l and 30 μ l), both root (RT) and leaf (LF) extracts showed minimal effect on seed mass preservation, likely due to insufficient insect control. However, at the effective doses of 60 μ l and 80 μ l, the root extract treatments resulted in a significant reduction in weight loss (compared to the untreated control, which would show maximal loss), suggesting a potent protective effect. This preservation is a direct consequence of the extracts' ability to eradicate the insect population and prevent post-embryonic development. While the leaf extracts showed less efficacy in preserving seed mass at the 60 μ l dose, both extracts ultimately converged on a similar protective outcome at the 80 μ l dose, although the root extract generally maintained a more pronounced effect.

3.9 The germinative capacity of seeds

To ensure the extracts are safe for seed viability, a final test assessed the germinative capacity of treated seeds (Graph 5). The goal was to rule out phytotoxicity and confirm the maintenance of seed quality.



(RT: root ; LF: leaf ; SBV : Sambava ; TDD : Tsiroanomandidy ; FNR : Fianarantsoa)

Graph 5. The germinative capacity of seeds

The results confirm that at low to medium doses (10 μ l, 30 μ l and 60 μ l), neither the root nor the leaf extracts exerted a significant negative effect on seed germination, suggesting they are not phytotoxic at effective biocidal concentrations. Furthermore, at the highest dose (80 μ l), the root extract exhibited an indirect positive effect on germination. This outcome is not due to a stimulatory property of the extract, but rather to its superior insecticidal action, which successfully inhibited insect growth and damage, thereby preserving the intrinsic germinative potential of the seeds. The leaf extract showed no such preservation effect on germination, reflecting its failure to control the infestation and prevent significant seed damage.

IV. Conclusion

This investigation rigorously evaluated the bioinsecticidal efficacy of methanolic root and leaf extracts from *Vetiveria zizanioides* against the destructive cowpea weevil, *Callosobruchus maculatus*. The findings unequivocally validate the root extract (RT) as a potent, multi-modal biopesticide, demonstrating significantly superior activity compared to the leaf extract (LF) across all assays.

In terms of direct toxicity, the root extracts displayed powerful fumigant and contact actions. Complete adult mortality (100%) was achieved with the root extracts at 60 µl/l air volume within 72 hours of fumigation. Notably, the 80 µl dose of the Tsiroamandidy root chemotype exhibited remarkable speed, reaching 100% mortality in just 48 hours via contact exposure, a result confirmed by highly significant differences ($P=0$).

Furthermore, the extracts were highly effective at disrupting the pest's reproductive cycle. Fertility was entirely suppressed by root extract doses of 60µl and higher, supported by a highly significant Dunnett test result ($P=0.000$). Most impressively, the root extracts demonstrated absolute ovicidal activity, resulting in a zero hatching rate even at the minimum dose of 10µl, compared to 82% in the untreated control.

Complementing the lethality, the root extracts proved to be effective protectants, classified as Repellent (Class IV) at doses 60µl (averaging 60.71% repulsion). Crucially, this protective action resulted in the preservation of seed germinative capacity, confirming that the extract is non-phytotoxic and exerts an indirect positive effect on seed quality by eliminating the pest. These data collectively substantiate the promising potential of *Vetiveria zizanioides* root extract as a sustainable and highly effective botanical alternative for stored cowpea protection.

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