



Potency of *Dioscorea sansibarensis* (Dioscoreaceae) Leaf Eluates on *Callosobruchus chinensis*, Linnaeus 1758 (Coleoptera: Bruchidae) in the Protection of *Phaseolus vulgaris* (Fabaceae)

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Abstract

Post-harvest losses of stored *Phaseolus vulgaris* to the bean weevil *Callosobruchus chinensis* have reached levels of significant concern. Governments and health organisations propose the discovery of reliable, healthy and biodegradable pesticides with higher selectivity and a broad spectrum. This study presents investigations on the activities of *Dioscorea sansibarensis* leaf extracts on the mortality of *C. chinensis* and reduction of their egg-laying ability. Laboratory experiments under Completely Randomized Design (CRD) were carried out to determine the mortality and anti-oviposition activity of the vacuum liquid chromatography (VLC) eluates of *n*-hexane (HE), chloroform (CE), ethyl acetate (EE), *n*-butanol (BE) and methanol (ME) at different concentrations. Bioassay data were subjected to nonparametric statistical analysis and a generalized linear model at $p = 0.05$. Statistical results showed that the VLC eluates had a mortality activity of 88.01% ($R^2 = 0.8801$). Treatment by 0.025 g of HE and 0.075 g of CE had 9.60 and 11.50, respectively at $p = 0.181$. These mortality records were high as to 0.1 g of ME, 0.05 g of EE and 0.075 g of BE that recorded 8.55, 8.45 and 7.80, respectively. Treatments by 0.05 g of CE, 0.025 g of HE, 0.05 g of EE and the positive control recorded mortality of 10.50, 9.60, 8.45 and 8.35, respectively. The highest mortality was observed in the treatment by 0.075 g of HE and 0.1 g of HE with 12.85 and 13.70, respectively at $p = 0.377$. The VLC eluates had an anti-oviposition activity of 24.98% ($R^2 = 0.2498$) on the *C. chinensis*. The generalized linear model reported Wald Chi-Square values of 4.363; $p = 0.037$, 0.711; $p = 0.399$, 9.125; $p = 0.003$, 4.363; $p = 0.037$ on the treatment by 0.025 g of CE, 0.05 g of EE, 0.075 g of BE and 0.1 g of ME, respectively. At $p = 0.051$, 0.1 g of CE and the positive control attained oviposition of 89.25 and 96.75 respectively. The study presents the first documentation of the lethal activity of *D. sansibarensis* on the *C. chinensis* pulse beetle. This could help in the development of Integrated Pest Management (IPM) and could help in the elimination or suppression of the infestation.

Keywords: anti-oviposition, *Callosobruchus chinensis*, *Dioscorea sansibarensis* leaf, mortality, *Phaseolus vulgaris*, vacuum liquid chromatography (VLC) eluates.

Introduction

In recent years, losses of stored *Phaseolus vulgaris* (common bean) to *Callosobruchus chinensis* LINNAEUS, 1758 (Coleoptera: Bruchidae) in the tropics have reached levels of significant concern (Mishra et al. 2018). An

increase in damage by the bruchids is due to farmers being mindful of the environmental and health hazards posed by synthetic pesticides and the ban of synthetic insecticides with undegradable toxins by the governments and health organisations (Iram et al. 2013).

These hazards have intensified the need to discover reliable, healthy and biodegradable pesticides with higher selectivity and a broad spectrum. Substitute approaches have led to re-evaluation and use of botanical insecticides either as crude or formulated extracts as control agents (Babarinde et al. 2018, Kishor and Tiwari 2021).

Plants are a rich source of natural products, and many plant species synthesize their chemicals and store them in different parts of the plants. Plant compounds are easy to extract using different solvents. Crude extracts of plants are fractionated using vacuum liquid chromatography (VLC) with various solvents with varying polarity and solubility and appear in the form of slurries, powder or shredded segments (Targett et al. 1979). Ethanol, ethyl acetate and chloroform VLC elute of *Melia azedarach*, and *Aphanamixis polystachya* were found to cause mortality and reduction in the number of laid eggs, and this was due to the presence of compounds with insecticidal properties (Odeyemi et al. 2008, Hernández et al. 2017, Uyi et al. 2020).

Research shows that different organs of a plant have different phytochemicals with different concentrations, hence may exert different effects on insect pests. Shukla et al. (2009) reported that methanol and petroleum ether crude eluates of leaf and rhizome of *Acorus calamus* (sweet flag), caused adult mortality and adversely affected the adult emergence of the *C. chinensis* (Mots). According to Bekele (2002), crude eluates from leaf, pod and bark of *Millettia ferruginea* (Hochest) using distilled water, petroleum ether, acetone, *n*-hexane, methanol and ethanol exhibited anti-oviposition of the *Sitophilus zeamais*.

According to Kumar et al. (2017), the bioactive compounds present in the vegetative parts of the *Dioscorea sansibarensis* plant are not well exposed even though all parts, i.e., leaf, underground tuber, aerial bulbil and stem are found to be toxic to the herbivores and the insects of the family Chrysomelidae. Studies by Price et al. (2016a) reported the presence of

the phytochemical compounds phytol, 1-epi-gurjunene, palmitic acid, ethyl palmitate, methyl palmitate, isophytol, 2-heptadecanone and α -selinene in *D. sansibarensis* leaf with antibacterial effects. This study presents investigations on the activities of *D. sansibarensis* leaf eluates by vacuum liquid chromatography technique on the mortality of *Callosobruchus chinensis* and reduction of their egg-laying ability.

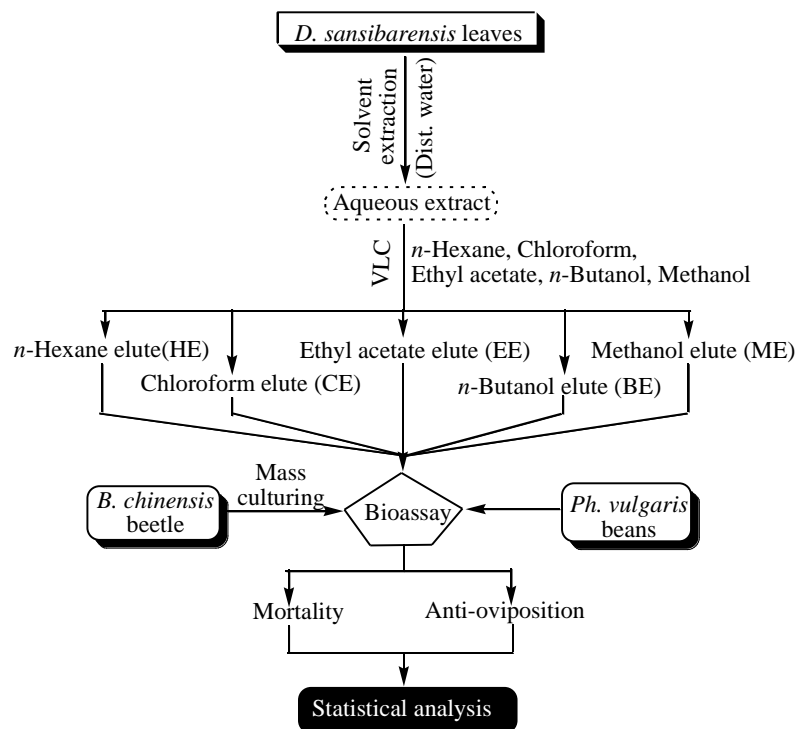
Materials and Methods

The materials and methods are summarised in Figure 1.

Preparation of materials

Fresh leaves of *D. sansibarensis* were identified and harvested in April 2019 from the Zoology-Botany Forest thickets at the University of Dar es Salaam located at 6° 45'0" S by 39° 15'0" E, altitude 80 m above sea level. Morphological identification was done at the Botany Department's herbarium, University of Dar es Salaam, where voucher specimen bearing reference number FMM 3910a was deposited. The plant material was properly washed with tap water, rinsed with distilled water and left to dry under shade in a well-ventilated area in the Entomology Laboratory of the Department of Zoology and Wildlife Conservation, University of Dar es Salaam.

Dry *D. sansibarensis* leaves were macerated and stored separately in airtight containers and stored at room temperature for experimental use. Healthy and undamaged *Ph. vulgaris* bean seeds were purchased directly from Kisarawe farm (7° 10'0" S, 38° 50'0" E and altitude of 252 m above sea level) after harvest. The seeds were sun-dried and stored at room temperature with 10 ± 2% moisture content for experimentation. The analytical grade solvents; *n*-hexane, chloroform, ethyl acetate *n*-butanol, methanol for use in vacuum liquid chromatography (VLC) and the standard synthetic pesticide; Actellic 50 EC (500 g/L AI, 100 g/90 kg Syngenta, Switzerland) were purchased from Chem equip laboratory suppliers in Tanzania.



Key:
VLC - Vacuum liquid chromatography

Figure 1: Summary of the study methodology.

Vacuum liquid chromatography of plant materials

From the powder of *D. sansibarensis* leaves, 110 g was weighed and soaked in 500 mL of methanol at 27 °C for two days. The solution was percolated through Whatman no. 1 filter paper then concentrated to dryness at 40 °C. The dry methanol extract was weighed and subjected to fractionation in vacuum liquid chromatography (VLC) using *n*-hexane, chloroform, ethyl acetate, *n*-butanol and methanol solvents. The VLC eluates were collected separately as fractions and dried using a rotary evaporator at 40 °C (Targett et al. 1979).

Preparation of working concentrations

Working solutions with different concentrations were prepared by diluting 0.025, 0.05, 0.075 and 0.1 g of VLC elute of the *D.*

sansibarensis leaves in 5 mL of their respective fractionating solvents to give respective concentrations of 0.036, 0.072, 0.108 and 0.144 mg/mL. Following the manufacturer's instructions, Actellic 50 EC was used as the standard positive control by diluting 2.5 g in 5 mL of water to give a concentration of 0.02 mg/mL. These doses were applied on 50 g of *Ph. vulgaris* beans for bioassays. The bean seeds to be used as negative control (untreated seeds) were cleaned and dried in a hot air oven at 60 °C for 1 hour.

Stock culture of *Callosobruchus chinensis*

In a glass jar (10.3 cm wide and 15 cm depth) containing 350 g of dry and undamaged *Ph. vulgaris* beans, approximately 200 females and males of *C. chinensis* were placed and the mouth was covered with a muslin cloth. The jar was incubated for 1 week in darkness at 28 ± 2

°C and relative humidity of $65 \pm 5\%$ to facilitate egg-laying by the *C. chinensis*. The bruchids were removed and the beans were incubated for mass culturing of the F_1 progeny of *C. chinensis*. The pure culture of the F_1 breed was used in experimentation.

Assays for adult mortality

Fifty grams (50 g) of clean, disinfected common bean seeds were weighed into different sterilized glass bottles and mixed properly with VLC extracts of the leaf. Ten pairs of newly emerged adult weevils were introduced into the treated legumes in glass bottles and covered with a muslin cloth. Each treatment was replicated four times and arranged in a Completely Randomized Design (CRD) on a laboratory bench. Mortality was determined by the number of dead insects which were recorded every 24 hours for ten days and computed using the formula adopted by Hasan et al. (2020).

Assays for anti-oviposition

Ten pairs of *C. chinensis* beetles were subjected to glass jars containing treated seeds and controls. The containers were incubated in the dark at $28 \pm 2^\circ$ C with a relative humidity of $65 \pm 5\%$ for egg-laying by the weevils. On the 5th day of incubation, all weevils, dead or alive, were removed from each container. Eggs laid on the bean seeds were counted with the aid of a dissecting microscope and computed using the formula adopted by Mauti et al. (2020).

Statistical analyses

Mortality and anti-oviposition analyses were performed using the SPSS software (version 20). The analysis involved the determination of Poisson distribution by nonparametric (Kolmogorov–Smirnov) test and descriptive statistics of the mean-variance. The Poisson distributed data from the mortality and anti-oviposition were subjected separately to generalized linear model analysis at $p = 0.05$.

In the model of analyzing the mortality and anti-oviposition, the concentrations of the doses were the independent variables as data of mortality and anti-oviposition were the dependent variable.

Results

Adult mortality of *C. chinensis*

Tabulated results of the lowest dosage showed mortality was high in the treatment by hexane elute, i.e., 0.025 g of HE, CE and EE recorded mortality of 3.0, 1.75 and 0.50, respectively as 0.025 g of BE and ME had no mortality effect on the bruchids (Table 1). Data subjected to statistical analysis ($N = 440$) showed a Poisson distribution; the Kolmogorov-Smirnov test recorded 4.441 and the descriptive statistics recorded a mean of 8.49 with a variance of 31.417. Statistically, mortality increased with days of exposure, i.e., harmonic mortality on the 1st day, 2nd, 3rd, 4th and 5th day were 3.30, 5.99, 8.40, 11.25 and 13.93, respectively. The VLC eluates had a mortality activity of 88.01% ($R^2 = 0.8801$) on the *C. chinensis* where the treatment by 0.025 g of HE and 0.075 g of CE had 9.60 and 11.50, respectively at $p = 0.181$. These mortality records were high as to 0.1 g of ME, 0.05 g of EE and 0.075 g of BE that recorded 8.55, 8.45 and 7.80, respectively.

The generalized linear model reported a Wald Chi-Square value of 1.022; $p = 0.312$, 0.630; $p = 0.427$, 1.453; $p = 0.228$ and 0.813; $p = 0.367$ in the treatment by the positive control, 0.025 g of HE, 0.05 g of CE and 0.05 g of EE, respectively. Treatments by 0.05 g of CE, 0.025 g of HE and 0.05 g of EE recorded mortality of 10.50, 9.60 and 8.45, respectively which were high to the positive control that had 8.35. The highest mortality was observed in the treatment by 0.075 g of HE and 0.1 g of HE with 12.85 and 13.70, respectively at $p = 0.377$.

Table 1: Mortality of *C. chinensis* reared for 5 days on *Ph. vulgaris* treated with VLC eluates of *D. sansibarensis* leaves

VLC eluates of <i>D. sansibarensis</i> leaves	Conc. (g/50 g of beans)	Mortality at days after treatment (Mean ± S.E.)				
		Day 1	Day 2	Day 3	Day 4	Day 5
<i>n</i> -Hexane elute (HE)	0.025	3.0 ± 0.41 ^w	6.0 ± 0.40 ^s	9.25 ± 0.48 ^l	13.0 ± 0.71 ^e	16.75 ± 0.48 ^b
	0.05	5.0 ± 0.82 ^u	8.0 ± 0.91 ⁿ	12.0 ± 0.41 ^f	16.0 ± 1.08 ^d	19.0 ± 0.40
Chloroform elute (CE)	0.075	7.0 ± 1.08 ^q	10.75 ± 0.85 ^h	14.0 ± 0.41 ^d	17.0 ± 0.71 ^b	19.75 ± 0.25 ^a
	0.1	7.75 ± 1.25 ⁿ	12.25 ± 0.85 ^f	14.75 ± 0.75 ^d	17.75 ± 0.85 ^b	20.0 ± 0.00 ^a
	0.025	1.75 ± 0.85 ^z	3.25 ± 0.48 ^w	6.0 ± 0.58 ^s	9.0 ± 0.41 ^m	13.0 ± 0.91 ^e
Ethyl acetate elute (EE)	0.05	5.25 ± 0.85 ^t	7.50 ± 0.87 ^o	10.75 ± 0.85 ^h	13.0 ± 0.82 ^e	16.0 ± 1.08 ^d
	0.075	6.0 ± 0.71 ^s	9.0 ± 0.41 ^m	12.0 ± 0.71 ^f	14.25 ± 0.63 ^d	16.25 ± 0.85 ^c
	0.1	7.0 ± 0.82 ^q	9.75 ± 0.48 ^j	13.75 ± 0.85	15.75 ± 0.75 ^d	17.0 ± 0.91 ^b
<i>n</i> -Butanol elute (BE)	0.025	0.50 ± 0.50 ^z	2.0 ± 0.41 ^y	4.25 ± 0.85 ^v	8.75 ± 1.03 ^m	13.0 ± 0.91 ^e
	0.05	2.25 ± 0.48 ^x	5.75 ± 1.11 ^s	9.25 ± 1.31 ^l	11.0 ± 1.22	14.0 ± 0.82 ^d
	0.075	5.75 ± 1.25 ^s	8.50 ± 0.96	11.75 ± 1.03	13.25 ± 1.31 ^e	16.0 ± 1.47 ^d
Methanol elute (ME)	0.1	5.75 ± 0.85 ^s	9.75 ± 1.25 ^j	13.0 ± 1.02 ^c	16.0 ± 0.82 ^d	19.75 ± 0.25 ^a
	0.025	0.00 ± 0.00	0.75 ± 0.48 ^z	2.75 ± 0.48	6.25 ± 1.11 ^r	10.0 ± 0.71 ⁱ
	0.05	0.50 ± 0.29 ^z	3.0 ± 0.41 ^w	6.0 ± 1.08 ^s	9.50 ± 0.65 ^k	12.25 ± 1.11 ^f
Actellic 50 EC	0.075	3.25 ± 0.63 ^w	5.25 ± 0.48 ^t	7.50 ± 0.65 ^o	10.0 ± 0.41 ^l	13.0 ± 1.08 ^e
	0.1	4.25 ± 0.85 ^v	6.25 ± 0.85 ^r	9.0 ± 0.82 ^m	12.75 ± 1.11 ^f	14.25 ± 0.85 ^d
	0.025	0.00 ± 0.00	0.00 ± 0.00	2.0 ± 0.41 ^y	5.0 ± 0.91 ^u	7.25 ± 1.03 ^p
Negative control	0.05	0.25 ± 0.25 ^z	2.0 ± 0.41 ^y	4.0 ± 0.41	6.75 ± 0.48	9.25 ± 0.85 ^l
	0.075	2.0 ± 0.71 ^y	3.75 ± 0.84	6.50 ± 0.65	9.50 ± 0.65 ^k	9.25 ± 2.78 ^l
	0.1	3.0 ± 0.82 ^w	5.0 ± 1.08 ^u	9.0 ± 1.08 ^m	11.50 ± 0.65 ^g	14.25 ± 0.85 ^d
Negative control	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.25 ± 0.25

Treatment with Poisson distributed data was subjected to a generalized linear model. The means in the table that do not share a letter are significantly different (N = 440; $p \leq 0.05$).

Anti-oviposition activity of VLC eluates of *D. sansibarensis* leaves against *C. chinensis*

Graphic results showed that 0.025 g of ME had an anti-oviposition of 457.5 g and HE recorded 142.75 g (Figure 1). When the eggs laid on the treated seeds are equated to the eggs laid on the negative control (untreated seeds), 0.025 g of ME, EE, CE and HIM an anti-oviposition of 25.15%, 41.92%, 51.69% and 76.65%, respectively. 0.1 g of ME and 0.025 g of CE showed a similar anti-oviposition of 57.51%

(Figure 2). Data subjected to statistical analysis (N = 88) showed a Poisson distribution; the Kolmogorov-Smirnov test recorded 4.313 and the descriptive statistics recorded a mean of 239.53 with a variance of 22351.148. The VLC eluates had an anti-oviposition activity of 24.98% ($R^2 = 0.2498$) on the *C. chinensis*. The generalized linear model reported Wald Chi-Square values of 4.363; $p = 0.037$, 0.711; $p = 0.399$, 9.125; $p = 0.003$, 4.363; $p = 0.037$ on the treatment by 0.025 g of CE, 0.05 g of EE, 0.075 g of BE and 0.1 g of ME, respectively.

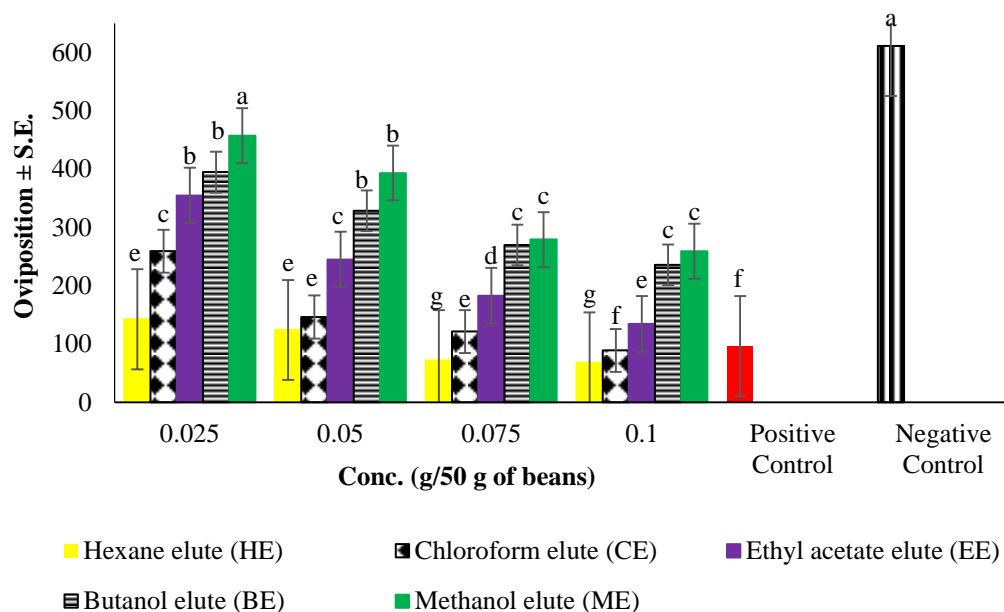


Figure 2: Oviposition by *C. chinensis* on *Ph. vulgaris* seeds treated with vacuum liquid chromatography (VLC) eluates of *D. sansibarensis* leaves. Means in the graph that do not share a letter are significantly different ($N = 88$; $p \leq 0.05$).

Treatment by 0.075 g of CE and 0.025 g of HE had oviposition of 121.5 g and 142.8, respectively at $p = 0.103$. These oviposition results were comparatively lower to 163.0, 259.3 and 236.0 obtained by 0.075 g of EE, 0.1 g of ME and 0.1 g of BE, respectively. At $p = 0.051$, 0.1 g of CE and the positive control attained oviposition of 89.25 and 96.75, respectively which were higher than the eggs counted in the treatment by 0.075 g of HE (72.25). A recommendable anti-oviposition effect was displayed by the hexane extract as observed in the treatment by 0.1 g of HE (32.50) as the lowest anti-oviposition effects were recorded by the methanol extracts.

Discussion

The results in this study showed that *D. sansibarensis* leaves were effective by causing mortality, anti-oviposition and reduced emergence of the F_1 progeny from the bean weevils. These results were similar to the findings reported by Mauti et al. (2019a) on the

essential oil derived from *D. sansibarensis* leaves that caused mortality, anti-oviposition and reduced the number of emerging *C. chinensis* weevils from *Ph. vulgaris* bean seeds. Various researches have shown that the leaf of a plant is a repository of compounds with diverse functions and activities on bean weevils including mortality and prevention of egg-laying on the common beans (Uyi et al. 2020).

The aromatic and antifeedant nature of VLC elute of hexane of *D. sansibarensis* to leaf-feeding insects of the families Chrysomelidae, Curculionidae and Meloidae suggest that it may contain constituents that are highly effective against bean weevils. Terpenoids, flavonoids and saponins identified by thin-layer chromatography in *D. sansibarensis* were responsible for the anti-oviposition and Inhibition of F_1 emergence of *C. chinensis* (Mauti et al. 2020). According to Mauti et al. (2019b) and Philbert (2021), the common factors affecting the bioactivity and

properties of a compound is the part of a plant that is extracted and the solvent used for extraction. Volatile and active compounds in plants extracted by hexane solvent have shown deterrent and toxic properties to *C. chinensis* weevils (Bubalo 2018).

In this study, *n*-hexane was used as a non-polar solvent and its elute of the *D. sansibarensis* leaves portrayed deterrent activities in mortality and anti-oviposition of *C. bruchus*. This is similar to the findings by Danga et al. (2015), which revealed the hexane eluates of *Callistemon rigidus* leaves showed mortality and anti-oviposition of *Callosobruchus maculatus* on cowpea seeds as compared to the chloroform, ethyl acetate and methanol eluates.

Results obtained for hexane elute of *D. sansibarensis* in this study agree with previous results recorded by Shukla et al. (2009) on the hexane extract of *A. calamus*, whereby a dose of 0.04 g caused 92.4% of mortality and 93.5% inhibition of oviposition on chicken pea seeds. These results were slightly encouraging compared to methanol extracts of *A. calamus* leaf. A study by Javaid et al. (2020) on the hexane elute of *Cenchrus setigerus* leaves applied on cowpea seeds recorded a 25% mortality within 24 hours and 87.25% of anti-oviposition by *C. maculatus*.

In this study, chloroform eluates of the *D. sansibarensis* leaves showed mortality of the *C. chinensis* beetle, this was contrary to findings of studies made by Mondal and Islam (2008) on *C. maculatus*. They recorded no mortality was observed in treatments by chloroform extract of *Derris indica* leaves; this difference might have been influenced by the phytochemical constituents presents in *D. sansibarensis* and absent in *D. indica*. Observation on the methanol elute of *D. sansibarensis* leaves showed low mortality of the beetle. This finding was similar to results obtained by Mustary et al. (2020) on *Bruchus pisorum* using methanol extract of *Artocarpus heterophyllus* leaves where the methanol eluates showed mortality properties. The similarity in results might be due to the

presence of similar phytochemical compound(s) with similar composition(s) more so, using a precise solvent in the extraction of the compound(s). Based on the findings in the present investigations, it is recommended that the exploitation of the hexane eluates of *D. sansibarensis* leaves in the development of Integrated Pest Management (IPM) is a feasible control method in elimination or suppression of the infestation of bruchids.

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Declaration of interest

The author declares no potential conflict of interest.

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