

**Evaluation of the repellent effect of *Dioscorea sansibarensis* PAX  
(Dioscoreaceae) leaf essential oil against *Bruchus chinensis*  
LINNAEUS, 1758 (Coleoptera: Bruchidae)**

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**ABSTRACT.** Laboratory experiments were conducted to determine the repellent activities of the ornamental plant *Dioscorea sansibarensis* leaf essential oil against the *Bruchus chinensis* bean weevil in the protection of stored legumes. The leaves exhibited potential repellency in a Y-tube olfactometer. The tabulated data show that 10 µL of leaf oil exhibited a repellency of 41.33%, with a grouped median of 53.33%, which was better than 10 µL of Actellic 50 EC, which had a repellency of 36.00% and a grouped median of 40%. Statistically, there was no significant difference in the percentage repellency obtained from the leaf oil and Actellic 50 EC at different concentrations (10, 20, 30 and 40 µL/mL,  $X^2(7, N=200) = 114.93, P < 0.05$ ). The GC-MS analysis identified 16 compounds in the leaf oil. The main compounds and their percentage composition were phytol (19.46%), 1-epi- $\alpha$ -gurjunene (11.71%), palmitic acid (10.48%), ethyl palmitate (8.87%), methyl palmitate (7.72%), isophytol (5.99%), 2-heptadecanone (4.59%) and  $\alpha$ -selinene (4.5%). The repellency caused by the leaf oil may have been due to the presence of  $\alpha$ -selinene, also known as naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1S-(1*a*.,7*a*.,8*a*.)]-. *Dioscorea sansibarensis* may be a good choice for repellent formulations.

**KEY WORDS:** *Bruchus chinensis*, *Dioscorea sansibarensis* leaves, essential oil, Y-tube olfactometer, repellency.

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## INTRODUCTION

*Bruchus chinensis* LINNAEUS, 1758 (Coleoptera: Bruchidae) is also known as *Callosobruchus chinensis* (LINNAEUS, 1758) and the Chinese bruchid (MUKERJI & BHUYA 1937). *Bruchus chinensis* breeds in every continent except Antarctica and is common in the tropical and subtropical regions of Asia, Africa, and Central and South America (KUMAR & KUMAR 2018). This pest has a close association mainly with leguminous plants, where it damages seeds (beans), both in the field and in stores. The beetle lays its eggs on the seed surface, from where the newly-hatched larvae bore into the seed. Pupation takes place inside the bean seed, from which the adult emerges (HONG et al. 2017).

*B. chinensis* management is usually based on a range of synthetic pesticides in the form of gaseous fumigants, solutions and residual insecticides (TRIPATHI 2018). Actellic 50 EC mixed with water is sprayed onto seeds and/or storage sacks, or fumigated on the inside walls of silos and granaries to prevent attack by *B. chinensis* beetles (NGOWI et al. 2007). Current research has shown that the Actellic 50 EC residues which remain on the seeds may have adverse effects on humans and the environment in the form of biomagnification and toxicity to non-target organisms. Persistent usage of Actellic 50 EC has also been found to increase resistance to the target insects (MASSALATCHI et al. 2017). Finding and developing alternative insecticides from botanicals as a strategy for sustainable insect pest management in agriculture is crucial. They are convenient to use, since they are biodegradable, environmentally-friendly and safe. Hence, they are potentially suitable for use as pure compounds or in an integrated form, as some are specific while others can have multiple target sites (KARANI et al. 2017, CAMPOS et al. 2018).

Plants contain secondary metabolites, which on the one hand protect them from being eaten by herbivores and from being infected by microbial pathogens, and on the other serve as attractants for pollinators and seed-dispersing animals. These characteristics have imparted different properties to plants, as a result of which they have medicinal, antimicrobial and insectivorous uses, among others (SABIHA et al. 2017). Natural insecticides of plant origin have been investigated in the form of powders and slurries, ashes, aqueous/solvent extracts and oils. In some cases, plant extracts exhibit repellent activity when they are mixed with seeds; in others, they attract and kill storage pests when these ingest them (UPADHYAY & AHMAD 2011, CAMPOLO et al. 2018).

Although there are numerous reports on the repellent activities of plant oils on bruchids, fewer data have been published regarding their repellency against *B. chinensis*. Plant essential oil from sweet flag (*Acorus calamus* L.) has been found to have repellent chemosterilant properties against *Collasobruchus maculatus* (FABRICIUS, 1775) (JAYAKUMAR et al. 2017). Oils from cinnamon (*Cinnamomum zeylanicum* J.PRESL), clove (*Syzygium aromaticum* (L.) MERRILL & PERRY), rosemary (*Rosmarinus officinalis* L.),

bergamot (*Citrus bergamia* RISSO) and Japanese mint (*Mentha arvensis* L.) have repellent activities against *Bruchus dentipes* (BAUDI, 1886) (TRIVEDI et al. 2017). CHILUWAL et al. (2017) reported repellency against *B. chinensis* by oils from Star anise (*Illicium verum* HOOK. F.), Linaloe berry (*Bursera delpechiana* POISS.), Croton (*Croton anisatum* BAILL.), Chinese cassia (*Cinnamomum cassia* (L.) J.PRESL) and Brazilian rosewood (*Aniba rosaeodora* DUCKE).

The *Dioscorea sansibarensis* PAX liana, native to Tanzania, is one of the largest and most widely distributed species of the genus *Dioscorea* L. in the coastal zones. The climber produces both bulbils and underground tubers, which several researchers have characterized as toxic. Despite its vegetative parts being evergreen and the leaves broadly and openly distributed, they are undamaged by chewing insects and destructive herbivores avoid feeding on them. The leaves of the plant have a characteristic scent when crushed into a paste. Traditionally *D. sansibarensis* has been used for medicinal purposes and of late it has gained pharmaceutical interest (BURKILL 1985, PRICE et al. 2016, KUMAR et al. 2017). The present study focuses on assessing the repellent potency of the essential oil retrieved from the leaves of *D. sansibarensis* against the notorious pest *Bruchus chinensis* and to analyse the compounds present using GC-MS.

## MATERIALS AND METHODS

### Plant material

The leaves of *D. sansibarensis* were collected in the Zoological-Botanical forests of the University of Dar es Salaam (6° 45'0" S, 39° 15'0" E, altitude 80 m above sea level) and taken to the Entomology Laboratory of the Department of Zoology and Wildlife Conservation at the University of Dar es Salaam, Tanzania. Morphological identification was performed at the herbarium of the Botany Department of the same institution, where the voucher with the reference number FMM 3910a is deposited.

### Extraction of *D. sansibarensis* leaf essential oil

Freshly collected *D. sansibarensis* leaves were thoroughly washed with tap water, rinsed with distilled water and left to dry in the shade in a well-ventilated area for 5 days at room temperature. After drying, 250 g of leaves were macerated and homogenized using an electric grinder in the laboratory. The oil was extracted from the homogenized leaves by hydro-distillation using a Clevenger type apparatus for 4 hours to yield a water and oil mixture. The oil was separated from water by solvent-solvent extraction using diethyl ether in a separating funnel, and concentrated under vacuum in a rotary evaporator at

a temperature of 40 °C. The oil was stored in a refrigerator at 4 °C and subsequently used in the bioassays.

#### **Analysis of compounds using gas chromatography coupled to mass spectrometry**

The identity of the essential oil constituents was established by GC-MS performed on a Hewlett-Packard HP 5973 mass spectrometer interfaced with an HP 6890 gas chromatograph. The mass selective detector was operated in electron impact mode with an ionization energy of 70 eV and a mass range from  $m/z$  40-380. An HP-5 column (30 m long and 0.25 mm internal diameter with a film thickness of 0.25  $\mu$ m) was initially kept at a temperature of 60 °C, then gradually heated to 250 °C at 5 °C /min intervals. The carrier gas was helium (99.9%). In some cases where the separation of peaks was inadequate, the temperature and programme rate were adjusted. Each sample was dissolved in hexane to give a 1% w/v solution; 0.2  $\mu$ L of the oil sample was injected into the GC-MS equipment at a temperature of 250 °C, from which the gas chromatograms and mass spectra were obtained.

Mass spectral deconvolution and automated calculation of the retention index was performed by the automated mass spectral deconvolution and National Institute of Standards and Technology (NIST). Standard solutions of linear alkanes (C<sub>7</sub>-C<sub>30</sub>, Sigma-Aldrich 49451-U) were used for Kovats RI calibration in the GC-MS system. Data deconvolution was performed with the following specifications: component width = 12; adjacent peak subtraction = 2; resolution = low; sensitivity = very low; shape requirements = medium. Compounds were identified from the deconvoluted mass spectra by comparison, matching their spectra to those recorded in the Wiley 275 and NIST Mass Spectral Library with reference to the associated database and literature.

#### **Preparation of working solutions**

Working solutions with different concentrations were prepared by diluting 10, 20, 30 and 40  $\mu$ L of oil in 1 mL of acetone, while water was used as the diluting solvent for the doses serving as the positive control. These doses represented the respective concentrations of 0.036, 0.072, 0.108 and 0.144 mg/mL for leaf oil and 0.01, 0.02, 0.03 and 0.04 mg/mL for the positive control (Actellic 50 EC). The commercial synthetic pesticide Actellic 50 EC (500g/L AI, 100 g / 90 kg Syngenta, Switzerland) was purchased from agricultural input suppliers in Dar es Salaam for use as the positive control.

#### **Culturing of *Bruchus chinensis***

Healthy common bean (*Phaseolus vulgaris* L.) seeds were purchased directly from the farm after harvest. The seeds were thoroughly cleaned, sun-dried and stored with 10  $\pm$  2% moisture content. 1 kg of undamaged, healthy *P. vulgaris* beans were dried under direct

sunlight for 7 days, disinfested in an oven at 60 °C for 1 hour and placed in glass jars (27 cm height × 16 cm diameter). Approximately 400 adults of *Bruchus chinensis* beetles were released into the jar, the mouth of which was then covered with muslin cloth and secured with a rubber band. These jars were then incubated in darkness at  $28 \pm 2$  °C and  $65 \pm 5\%$  relative humidity (RH) for mating and oviposition for 7 days. Thereafter, the beetles were separated from the seeds by sieving and returned to their container for continual incubation. Incubation was continuous in order to maintain reproduction. Afterwards the emerged insects were used in the bioassays.

### Repellency assay

The repellent activity of the essential oil against *B. chinensis* was assessed using a Y-shaped olfactometer. The Y-tube was placed in the centre of a 23 x 16 x 11 cm black box, lined with black paper to prevent visual stimuli produced by the halogen lamp during illumination. The Y-tube olfactometer consisted of a “Y” shaped glass tube with three compartments (arms), each 10 mm in diameter and 10 cm in length. These compartments were the treatment arm, the control arm and the arm for introducing the beetle. The end of each arm was fitted with glass stoppers of appropriate sizes (3 cm in diameter). The stoppers had two narrow grooves allowing airflow into the olfactometer during the bioassays.

Filter paper discs (Whatman No. 1, of diameter 1 cm and weighing 140 mg) were dipped into the various dilutions of leaf oil and the positive controls (10, 20, 30 and 40 µL/mL) and allowed to dry in air. Acetone was used to sterilize the filter paper disc that was used as the negative control disc. The discs were inserted into their compartments, prior to the introduction of the weevils, and air-suction was applied at the Y-junction by means of an aspirator pump. This ensured that the olfactometer did not become saturated with the vapour from the oil, which was confined to the treatment arm of the olfactometer. The insects were induced by light and allowed to walk and decide which arm to move to at the “Y” shape bifurcation. All the air from the olfactometer to the aspirator pump was carried out away in a Tygon tube from the pump outlet.

For each assay, 10 pairs of weevils were selected and introduced into the olfactometer at once. Advantage was taken of the fact that the weevils are negatively phototactic by illuminating the introduction compartment of the weevils into the olfactometer with light from a 60 W bulb placed 15 cm away and screening the rest of the olfactometer in a carton. An average of 80-85% of the weevils was thus induced to migrate from the introduction arm to either the control or treatment arm during the period of the bioassay. The assay was left to run for one hour and then the number of weevils in the control arm (NC) and the treatment arm (NT) were counted. Percentage repellency (PR) values were computed using the formula:

$$\%PR = \{(NC - NT)/(NC + NT)\} \times 100$$

where: %PR: Percentage repellency

$N_C$ : Number of weevils in the control arm

$N_T$ : Number of weevils in the treatment arm

### Statistical analyses

All weevil repellency data were recorded, converted to percentages and the means compared by Duncan's New Multiple Range Test (DMRT) using the statistical program Minitab 2017 (version 18). One-way non-parametric ANOVA (Kruskal-Wallis H test) was used to determine the differences in the effectiveness of *Dioscorea sansibarensis* leaf oil and the positive control (Actellic 50 EC) at different concentrations (10, 20, 30 and 40  $\mu$ L/mL) using SPSS software (version 20), which included a normality test as well as homogeneity distribution and post-hoc analysis using Tukey's HSD test at  $P \leq 0.05$ . In this model, the treatment doses and exposure duration were the independent variables, and the percentage repellency was the dependent variable.

## RESULTS

### Constituents of *D. sansibarensis* leaf essential oil

*D. sansibarensis* leaves yielded  $0.26 \pm 0.02\%$  (w/w) oil, with a density of  $0.74 \pm 0.08$  g/mL. GC-MS analysis revealed a total of 16 different compounds, eluted by GC-MS analyser over a period of 30 minutes. The main components and their percentage compositions were phytol (19.46%), 1-epi- $\alpha$ -gurjunene (11.71%), palmitic acid (10.48%), ethyl palmitate (8.87%), methyl palmitate (7.72%), isophytol (5.99%), 2-heptadecanone (4.59%) and  $\alpha$ -selinene (4.5%) (Table 1).

### Repellency effect of *D. sansibarensis* leaf oil against *Bruchus chinensis*

*B. chinensis* beetles were subjected to an oil vapour test for 1 hour in the Y-tube olfactometer. The tabulated data show that *Dioscorea sansibarensis* leaf essential oil displayed repellent activity against *Bruchus chinensis*. 10  $\mu$ L of leaf oil exhibited 41.30% repellency, 10  $\mu$ L of the positive control 36% (Table 2). The sample size (n=200) was analysed for normality: the Shapiro-Wilk test showed a non-normal distribution of repellency except in the data relating to the treatments with 20  $\mu$ L of leaf oil, and 10 and 20  $\mu$ L of Actellic 50 EC. The non-parametric Levene test showed that the data was

**Table 1.** Kovats retention index, retention time and percentage chemical composition of the compounds identified in the GC-MS analysis of the oil extracted from *Dioscorea sansibarensis* leaves.

No.	RT <sup>a</sup>	Constituents	RI <sup>b</sup>	% <sup>c</sup>
1	21.49	$\alpha$ -Copaene	1375	2.95
2	22.12	1-Epi- $\alpha$ -gurjunene	1409	11.71
3	24.07	$\gamma$ -Muurolene	1477	2.69
4	24.31	Trans- $\beta$ -ionone	1489	2.44
5	24.48	$\alpha$ -Selinene	1493	4.50
6	26.78	1-Heptadecene	1692	2.05
7	29.18	2-Heptadecanone	1886	4.59
8	31.16	1-Nonadecene	1894	2.84
9	33.87	Methyl palmitate	1927	7.72
10	34.28	Isophytol	1947	5.99
11	34.77	Palmitic acid	1970	10.48
12	35.18	Ethyl palmitate	1993	8.87
13	37.37	Phytol	2113	19.46
14	38.76	Behenic alcohol	2468	2.39
15	43.73	Hexacosane	2600	1.41
16	46.69	Pentatriacontane	3500	1.66

<sup>a</sup> Retention time (minute); <sup>b</sup> the Kovats Retention Indices were calculated from our analysis with respect to a series of n-alkenes; <sup>c</sup> Percentage composition of a compound.

**Table 2.** Repellency (%  $\pm$  S.E.) by *D. sansibarensis* leaf oil against *Bruchus chinensis* using a Y-tube olfactometer.

Treatment	Concentration [ $\mu$ L/disc]			
	10	20	30	40
Leaf oil	41.3 $\pm$ 15.1 <sup>a</sup>	58.67 $\pm$ 7.72 <sup>b</sup>	68 $\pm$ 9.29 <sup>b,c</sup>	85.33 $\pm$ 7.12 <sup>d,e</sup>
Actellic 50 EC	36 $\pm$ 10.5 <sup>a</sup>	64 $\pm$ 9.09 <sup>b,c</sup>	74.67 $\pm$ 6.11 <sup>c,d</sup>	93.33 $\pm$ 2.11 <sup>e</sup>

Treatments with the same letters in the table are not statistically significantly different from each other. (N = 200), ( $P < 0.05$ ) using Tukey's HSD test.

heterogeneous at L(7, 199.27),  $P < 0.05$ . The Kruskal-Wallis H test indicated that there was no statistically significant difference in the percentage repellency obtained from the leaf oil and the Actellic 50 EC at different concentrations (10, 20, 30 and 40  $\mu$ L/mL,  $X^2(7, N=200) = 114.93$ ,  $P < 0.05$ ).

Statistically, 10  $\mu$ L of leaf oil had a grouped median of 53.3%, whereas 10  $\mu$ L of Actellic 50 EC had 40%. Post-hoc analysis showed homogeneity in 10  $\mu$ L of leaf oil and 10  $\mu$ L of Actellic 50 EC, which achieved respective repellencies of 41.33% and 36.00%. Treatments using 20  $\mu$ L of Actellic (64%), 30  $\mu$ L of Actellic (68%) and 30  $\mu$ L of leaf oil (74%) had a homogeneous effect on repellency against *B. chinensis* beetles. A homogeneity effect was also found with 40  $\mu$ L of leaf oil (85.33%) and 40  $\mu$ L of positive control (93.33%). Statistically, 20  $\mu$ L of leaf oil performed better than 10  $\mu$ L of Actellic 50 EC.

## DISCUSSION

Previous research on *Dioscorea sansibarensis* focused specifically on its medicinal applications; no such research to date has been based on its potential insecticidal, pesticidal or repellency properties. The fumigation studies carried out here showed that among the tested oils, *D. sansibarensis* leaf oils exhibited significant repellent activities. The leaf oil exhibited a repellent activity comparable to that of Actellic 50 EC. The results (Table 2) of this study showed that the oil was less effective at lower concentrations; however, larger doses of higher concentrations resulted in enhanced repellent activity. This corroborates the findings of NTONIFOR et al. (2010) that oils at different concentrations exhibited different repellency rates against Coleopterans.

The repellency elicited by the leaf oil may have been due to the presence of  $\alpha$ -selinene also known as naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1S-(1 $\alpha$ .,7 $\alpha$ .,8 $\alpha$ .)]- (4.5%). Naphthalene acts as a general arthropod fumigant against clothes moths and carpet beetles, a microbial inhibitor and an anthelmintic agent. The chemical is also used as a repellent against mammals and birds, i.e. bats, squirrels, rabbits, pigeons, sparrows and starlings (CHEN et al. 1998). Studies of the repellent activity of  $\alpha$ -selinene, a naphthalene derivative extracted from *Muscodor vitigenus* DAISY, STROBEL, EZRA & W.M. HES, exhibited repellency against wheat stem sawfly (*Cephus cinctus* NORTON, 1872). In an exposure of 7 minutes, 24 out of 30 *C. cinctus* flies responded by moving to the control arm. The same plugs showed repellent activity to *C. cinctus* flies after 3 weeks where 21 out of 30 flies were repelled by moving to the control arm of the Y-tube olfactometer (DAISY et al. 2002)

## CONCLUSION

These findings indicate the good potential of *Dioscorea sansibarensis* leaves as a repellent in the management of the bean weevil. Since plant-derived pesticides are

biodegradable and safer for higher animals, they offer a viable alternative to synthetic agrochemicals (KEDIA et al. 2015). Appropriate effective doses of *D. sansibarensis* leaf oil could save a resource-poor farmer from storage pest losses. Although this study has demonstrated the scientific rationale for the use of *D. sansibarensis* in ethnobotanical legume storage practices, further research on the chemistry and mechanism of action of the bioactive compounds extracted from *D. sansibarensis* is necessary.

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