

*Full Length Research Paper*

# **Repellent and insecticidal effects of essential oils of *Petroselinum crispum* (Mill.) Fuss and *Pimenta racemosa* var. *racemosa* (Mill.) J.W. Moore leaves on *Dinoderus porcellus* Lesne (Coleoptera: Bostrichidae)**

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Essential oils from leaves of *Petroselinum crispum* (Mill.) Fuss (Apiaceae) and *Pimenta racemosa* var. *racemosa* (Mill.) J.W. Moore (Myrtaceae) were evaluated for their repellent properties, insecticidal and fumigant activities against *Dinoderus porcellus* Lesne (Coleoptera: Bostrichidae). The essential oils of these plants were obtained by Clevenger-type hydro distillation method with yield of 0.47 and 1.09% w/w for *P. crispum* and *P. racemosa*, respectively. Essential oils of both plants exhibited a low repellent activity (class I and II of repellency) against *D. porcellus* at all tested concentrations. The *P. crispum* and *P. racemosa* essential oils were toxic to *D. porcellus* in contact, ingestion and fumigant assays. The *D. porcellus* adults were more susceptible to contact action of *P. crispum* (LD<sub>50</sub> = 1.15 µL/adult) than *P. racemosa* (LD<sub>50</sub> = 414.38 µL/adult) at 72 h, whereas in ingestion assay, *P. crispum* essential oil was the most effective toxin (LC<sub>50</sub> = 3.88 µL/g) at 21 days interval time. Strong feeding deterrence (68.97%) was achieved in *D. porcellus* adults by using *P. crispum* essential oil at a concentration of 0.4 µL/g of yam chips. In the fumigation assays, *P. crispum* (LC<sub>50</sub> = 7.22 µL/L air) essential oil was more toxic than *P. racemosa* (LC<sub>50</sub> = 3.39×10<sup>6</sup> µL/L air) against *D. porcellus* adults within 7 days. These findings, suggest that *P. crispum* essential oil was more active against *D. porcellus* adults and showed its potential for development as natural anti-feeding agent, and fumigant insecticide for managing *D. porcellus* adults in stored yam chips.

**Key words:** Contact toxicity, feeding deterrence, fumigant toxicity, repellent activity, storage insects.

## **INTRODUCTION**

Yam (*Dioscorea* species, Dioscoreaceae) is largely cultivated in West Africa for their starchy tubers, which contribute to food security in this region (Kiba et al., 2020). In Benin, fourth worldwide producer, yam contributes to 441 kcal/capita/day (FAO, 2018). However,

yam tubers have high water content, which makes them highly perishable causing severe losses which can reach 85% of stocks (Umogbai, 2013). Traditionally, yam tubers are processed and dehydrated on sun to obtain dried chips whose flour are cooked to obtain a very nourishing

dough (Omohimi et al., 2019). In northern Benin, yam chip flour is also used to prepare a type of couscous namely wassa-wassa, which is recommended to diabetic patients (Behanzin et al., 2018).

Unfortunately, insects attacked yam chips, cause important losses, reduce their market value and the quality of paste obtained with infested yam chips (Omohimi et al., 2019). Among these pests, *Dinoderus porcellus* Lesne (Coleoptera: Bostrichidae) is far the most abundant and damageable pest in stored yam chips (Loko et al., 2013). Currently, control of *D. porcellus* population is done by some farmers with synthetic insecticides (Loko et al., 2013) which lead to death of some persons (Adedoyin et al., 2008; Adeleke, 2009).

It is so urgent to find alternatives to synthetic insecticides for protection of stored yam chips. Potential alternatives to synthetic insecticides are plant-derived insecticides, which are less persistent in the environment, and often less toxic to mammalian (Smith and Thomas, 2020).

Essential oils exhibit various insecticidal and repellent properties. Due to their diverse biologically active compounds, the essential oils of parsley *Petroselinum crispum* (Mill.) Fuss and bay rum tree, *Pimenta racemosa* var. *racemosa* (Mill.) J.W. Moore have been suggested as suitable alternatives for controlling storage insect pests. Indeed, it is known from previous works that essential oils of *P. crispum* leaves have strong adverse effects on *Callosobruchus maculatus* (F.) (Massango et al., 2017), *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), and *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae) (Maroufpoor et al., 2016) as a toxic fumigant and reproductive inhibitor. Similarly, *P. racemosa* essential oils show a high toxicity against *Prostephanus truncatus* (Horn) (Noudogbessi et al., 2008) and *Tribolium castaneum* (Herbst) (Lee et al., 2002). However, the biological activities of essential oils of leaves of these plants have not been investigated yet on *D. porcellus*.

The composition of essential oils of parsley and bay-rum tree leaves from different origins has been studied by several authors. In general, the essential oils of parsley leaves have  $\beta$ -phellandrene, and 1,3,8-p-menthatriene as the main constituents (Petropoulos et al., 2004; Mulugeta et al., 2015; El-Zaeddi et al., 2016).

While essential oils of *P. racemosa* leaves composed of about twenty components with eugenol, myrcene, and chavicol as major components (Noudogbessi et al., 2008; Contreras-Moreno et al., 2016). The objective of this study was to test the possible repellent and insecticidal properties of essential oils of *P. crispum* and *P. racemosa* leaves against *D. porcellus*.

## MATERIALS AND METHODS

### Plant and extraction of essential oils

The fresh leaves of *P. crispum* and *P. racemosa* were collected in the town of Abomey-Calavi. The extraction was carried out using a Clevenger type apparatus at the Laboratory of Study and Research in Applied Chemistry (LERCA) of the Polytechnic School of Abomey-Calavi (EPAC) according to Saroukolai et al. (2014). Fresh leaves were subjected to hydrodistillation. Anhydrous sodium sulphate was used to remove water after extraction. Extracted oil was stored in airtight containers in a refrigerator at 4°C until experiment. The yield of essential oils (RD), defined as the ratio between the mass of essential oil obtained after the extraction (M') and the fresh or dry mass of the plant material used (M), was calculated according the formula:

$$RD(\%) = \frac{M'}{M} \times 100$$

### Insect culture

The adult of *D. porcellus* were collected from infested yam chips purchased at the Kpota market in the town of Abomey-Calavi. The rearing of *D. porcellus* was done according to the methodology described by Loko et al. (2020). Five hundred grams of healthy yam chips were infested with 50 adults of *D. porcellus* and maintained in the laboratory (temperature: 25 ± 2°C, RH: 70 ± 5%, and 12L/12D) (Oni and Omoniyi, 2012). F1 progeny were used for all experiments.

### Repellence bioassay

The method described by Babarinde et al. (2008) was used for testing repellence of essential oils of *P. crispum* and *P. racemosa* leaves against *D. porcellus*. The experimental design consisted of Petri dishes (9 cm diameter) containing two-half filter paper of Whatman No. 1. Four doses (5, 10, 15, and 20  $\mu$ L of stock solution dissolved in 0.2 mL of ethanol) of each essential oil was applied to a half paper disc (30 cm<sup>2</sup>). Each untreated half paper was joined to a treated using clear adhesive tape and place in Petri dish. The control experiment was to treat one paper half with pure ethanol (0.2 mL). After waiting for 10 min for the solvent to evaporate, 20 unsexed adults of *D. porcellus* (3-7 days old) were deposited in the centre of each Petri dish. The covered Petri dishes were placed in a dark room in the laboratory. The number of *D. porcellus* present on the control (C) and treated (T) filter papers was recorded after 30 min, 2, 4 and 8 h of exposure (Guruprasad and Akmal, 2014). Each treatment was replicated three times. The repulsion percentage (RP) was calculated using the formula:

$$RP = \left[ \frac{(C - T)}{(C + T)} \right] \times 100$$

The mean repulsion percentage of each treatment was assigned to the repellent classes: class 0 (RP ≤ 0.1%), class I (0.1 - 20%), class II (20.1 - 40%), Class III (40.1 - 60%), Class IV (60.1 - 80%) and Class V (80.1 - 100%). The bioactivity of each dose of essential oil

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was classified (values < 1 repellency; 1 neutral; > 1 attractant) according to the repellence indices (RI) calculated following the formula:

$$RI = 2PT / PT + PA$$

where PT = percentage of insects attracted by treated paper with essential oil and PA = percentage of insects attracted by paper treated with solvent.

### Contact toxicity by topical application

The contact toxicity of each essential oil was evaluated following the methodology described by Mukanga et al. (2010). Using a microsyringe, 1  $\mu$ L of the essential oils of leaves of each plant was applied at concentrations 0.1, 0.25, 0.5 and 1% (v/v) on the dorsal side of the thorax of 20 newly emerged adults (3-7 days old) *D. porcellus*. The treated insects were transferred individually in a plastic box containing 10 g of untreated yam chips. Solvent extracts were applied to control insects and each treatment was replicated four times. Insects were examined after 24, 48 and 72 h and the number of dead insects was recorded (Caballero-Gallardo et al., 2012).

The percentage of adult mortality was calculated and corrected according to Schneider-Orelli's formula (Püntener, 1981):

$$\text{Corrected mortality} = \frac{\% MT - \% MC}{100 - \% MC} \times 100 \quad (1)$$

where MT = mortality in the treatment, MC = mortality in the control.

### Ingestion toxicity and feeding deterrence

To evaluate the ingestion toxicity of each essential oil the methodology described by Loko et al. (2020) was used. For that, 10 g of yam chips were placed in plastic boxes (7 cm  $\times$  3.7 cm) and then treated with an acetone solution containing essential oils of *P. crispum* and *P. racemosa* leaves at different doses. The doses were prepared by diluting each time with 1 mL of acetone the respective volumes of 2, 4, 6, 8, and 10  $\mu$ L of essential oils. They were then dispersed homogeneously in the yam chips using a micropipette. Ten pairs of *D. porcellus* adults (3-7 days old) were introduced in plastic boxes containing treated yam chips. The tests were repeated 4 times for each essential oil, each dose and each control. The counts of dead insects were carried out at 1, 3, 5, 7, 14 and 21 days after exposure (Othira et al., 2009). The percentage of insect mortality was calculated using Schneider-Orelli's formula (Equation 1). The percentage of weight loss was calculated according to the formula:

$$\text{Weight loss (\%)} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100 \quad (2)$$

The feeding deterrence index (FDI) was calculated with FDI positive value corresponding to feeding deterrent effect and FDI negative value a feeding stimulant effect (Stefanazzi et al., 2011):

$$FDI(\%) = \frac{CC - CT}{CC} \times 100 \quad (3)$$

where CC = Consumption of control yam chips, CT = Consumption

of treated yam chips.

### Fumigant toxicity

The fumigant activity of essential oils of *P. crispum* and *P. racemosa* leaves against *D. porcellus* was evaluated using the methodology described by Liu and Ho (1999). 25 ml of each concentration (0, 5, 10, 20 and 40  $\mu$ L dissolved in acetone) of essential oil were applied on filter paper Whatman No. 1 (2 cm diameter) and placed on the underside of the screw cap of a glass vial (2.5 cm  $\times$  5.5 cm). After waiting 1 min for the solvent to evaporate, 10 adults of *D. porcellus* were introduced into the glass vial, which was subsequently closed tightly with the cap. Glass vials were placed in a dark room in the laboratory at ambient conditions. Each treatment was replicated four times and acetone was used as control. Insect mortality was observed daily for a week (Chu et al., 2012). The insect mortality and the weight loss were calculated using respectively Equations 1 and 2.

### Data analysis

The normality of mortality and repellence percentage data were tested before subjected to analysis of variance (ANOVA) or general linear model (GLM) using IBM SPSS Statistics version 25 software package. Data not following a normal distribution and not having homogeneity of variances were arcsine-transformed. Student Newman Keuls test was used to identify difference between treatments. Probit regression analysis was done on log-transformed data of various concentration-response experiments using Mathematica software version 11.3.0.0 (Wolfram Research Inc., Champaign, IL, USA). Lethal dose or concentration that kills or repels 50% of exposed insects (LD<sub>50</sub>, LC<sub>50</sub> or RD<sub>50</sub>) was obtained from derived regression equations.

## RESULTS

### Repellent activity

The results showed that *P. crispum* and *P. racemosa* essential oils acted as a repellent to *D. porcellus* adults, even at low concentrations (5  $\mu$ L/cm<sup>2</sup>) (Table 1). However, the repellence of both essential oils was not significantly different ( $P \geq 0.05$ ) at all corresponding doses. Repellent activity of *P. crispum* and *P. racemosa* essential oils on *D. porcellus* adults were not dose-dependent ( $P \geq 0.05$ ). In addition, there was no significant ( $P \geq 0.05$ ) interaction between oil concentration and exposure time on the repellence of both essential oils. After 30 min of exposure time, the essential oil of *P. crispum* at 5 and 10  $\mu$ L/cm<sup>2</sup>, showed a decrease of repellent effect with an exposure time increase (Table 1). However, with the high concentrations (15 and 20  $\mu$ L/cm<sup>2</sup>) of *P. crispum* essential oil, repellent effect had increased with exposure time. In general, the repellence of *P. racemosa* essential oil against *D. porcellus* decreased with increase of concentration. Results showed that *P. racemosa* essential oil possessed high repellent activity (54.6% after an 8-h exposure) against *D. porcellus* adults only at the lowest concentration of 5  $\mu$ L/cm<sup>2</sup>. Essential oils of *P. crispum* and *P. racemosa* at

**Table 1.** Repellence of *P. racemosa* and *P. crispum* essential oils on *D. porcellus*.

Plant	Concentrations ( $\mu\text{L}/\text{cm}^2$ )	Repellence (%) after				Mean	Class	Repellence index	Classification
		30 min	2 h	4 h	8 h				
<i>P. crispum</i>	5	36.7 $\pm$ 40.9 <sup>a</sup>	25.6 $\pm$ 22.5 <sup>a</sup>	21.6 $\pm$ 39.5 <sup>a</sup>	29.5 $\pm$ 11.8 <sup>a</sup>	28.3 $\pm$ 13.7 <sup>a</sup>	II	0.7 $\pm$ 0.1	Repellent
	10	22.4 $\pm$ 17.2 <sup>a</sup>	12.3 $\pm$ 11.7 <sup>a</sup>	-7.8 $\pm$ 11.1 <sup>a</sup>	19.6 $\pm$ 16.9 <sup>a</sup>	11.7 $\pm$ 7.1 <sup>a</sup>	I	0.8 $\pm$ 0.0	Repellent
	15	17.1 $\pm$ 21.2 <sup>a</sup>	38.9 $\pm$ 14.5 <sup>a</sup>	37.8 $\pm$ 29.6 <sup>a</sup>	53.5 $\pm$ 15.1 <sup>a</sup>	36.8 $\pm$ 9.8 <sup>a</sup>	II	0.6 $\pm$ 0.1	Repellent
	20	14.7 $\pm$ 20.4 <sup>a</sup>	30.3 $\pm$ 15.1 <sup>a</sup>	33.3 $\pm$ 33.9 <sup>a</sup>	43.7 $\pm$ 19.7 <sup>a</sup>	30.5 $\pm$ 10.7 <sup>a</sup>	II	0.7 $\pm$ 0.1	Repellent
<i>P. racemosa</i>	5	29.8 $\pm$ 10.6 <sup>a</sup>	42.6 $\pm$ 22.8 <sup>a</sup>	31.1 $\pm$ 28.9 <sup>a</sup>	54.6 $\pm$ 4.6 <sup>a</sup>	39.5 $\pm$ 8.8 <sup>a</sup>	II	0.6 $\pm$ 0.1	Repellent
	10	23.2 $\pm$ 10.6 <sup>a</sup>	10.4 $\pm$ 12.8 <sup>a</sup>	9.3 $\pm$ 32.9 <sup>a</sup>	27.9 $\pm$ 18.9 <sup>a</sup>	17.7 $\pm$ 9.1 <sup>a</sup>	I	0.8 $\pm$ 0.1	Repellent
	15	4.6 $\pm$ 9.5 <sup>a</sup>	12.5 $\pm$ 6.7 <sup>a</sup>	22.9 $\pm$ 23.3 <sup>a</sup>	9.4 $\pm$ 12.1 <sup>a</sup>	12.3 $\pm$ 6.4 <sup>a</sup>	I	0.8 $\pm$ 0.0	Repellent
	20	3.7 $\pm$ 3.7 <sup>a</sup>	20.7 $\pm$ 27.1 <sup>a</sup>	16.7 $\pm$ 38.4 <sup>a</sup>	26.9 $\pm$ 17.3 <sup>a</sup>	17.0 $\pm$ 11.3 <sup>a</sup>	I	0.8 $\pm$ 0.1	Repellent

There is no significant difference between the values in a column followed by the same letter ( $p < 0.05$ ).

**Table 2.** Dose of *P. racemosa* and *P. crispum* essential oils that repel 50% of exposed insects.

Essential oil	Exposure time	RD <sub>50</sub> ( $\mu\text{L}/\text{cm}^2$ )	Slope ( $\pm$ S.E.)	Intercept	$\chi^2$	P-value
<i>P. crispum</i>	30 min	2.39	-0.583 $\pm$ 0.35	5.219	7.68	0.768
	2 h	26.37	0.782 $\pm$ 0.25	3.871	1.43	0.013
	4 h	9.91	1.159 $\pm$ 0.17	3.847	8.54	0.000
	8 h	10.19	1.000 $\pm$ 0.20	3.980	2.22	0.047
<i>P. racemosa</i>	30 min	4.77	-2.405 $\pm$ 0.09	6.623	9.03	0.287
	2 h	5.62	-1.546 $\pm$ 0.13	6.155	6.49	0.138
	4 h	6.41	-1.515 $\pm$ 0.14	6.215	6.81	0.178
	8 h	8.44	-2.294 $\pm$ 0.09	7.095	6.40	0.007

CI: Confidence interval, S.E.: standard error,  $\chi^2$ : Chi-square.

various concentrations ranged in two class of repellency (class I and II). The repellent action (RD<sub>50</sub>) of *P. crispum* and *P. racemosa* essential oils on *D. porcellus* in function of exposure time is shown in Table 2. The RD<sub>50</sub> value indicates that essential oil of *P. crispum* at 30 min interval time was found to be significantly more repellent than that of *P. racemosa* with a RD<sub>50</sub> value of 2.39

$\mu\text{L}/\text{cm}^2$ . However, after 2, 4 and 8 h interval times, the RD<sub>50</sub> values of *P. racemosa* essential oil were lower than those of *P. crispum* (Table 2).

#### Contact toxicity

Essential oils of *P. crispum* and *P. racemosa* at diverse concentrations had a topical insecticidal

effect on *D. porcellus* (Table 3). The effect concentration of *P. crispum* and *P. racemosa* essential oils on the mortality of *D. porcellus* was not significant ( $P \geq 0.05$ ). However, contrary to *P. crispum*, the time of essential oil *P. racemosa* exposure had significant ( $P \leq 0.001$ ) effect on the mortality of *D. porcellus*. The interaction between oil concentration of both plants and exposure time

**Table 3.** Mortality of *D. porcellus* after topical application of *P. crispum* and *P. racemosa* essential oils and yam chips weight loss after 3 days of exposure.

Plant	Concentration (% v/v)	Corrected mortality (%)			Weight loss (%)
		24 h	48 h	72 h	
<i>P. crispum</i>	0.1	0.20 ± 3.63 <sup>a</sup>	6.68 ± 4.10 <sup>a</sup>	21.30 ± 1.87 <sup>a</sup>	16.08 ± 3.40 <sup>a</sup>
	0.25	5.00 ± 3.54 <sup>a</sup>	7.40 ± 7.58 <sup>a</sup>	15.08 ± 5.31 <sup>a</sup>	15.35 ± 2.70 <sup>a</sup>
	0.5	4.99 ± 3.73 <sup>a</sup>	14.72 ± 4.46 <sup>a</sup>	19.87 ± 5.21 <sup>a</sup>	22.48 ± 5.11 <sup>a</sup>
	1	7.70 ± 4.29 <sup>a</sup>	17.24 ± 6.06 <sup>a</sup>	19.59 ± 6.60 <sup>a</sup>	26.45 ± 5.58 <sup>a</sup>
<i>P. racemosa</i>	0.1	6.32 ± 1.23 <sup>a</sup>	0.00 ± 3.13 <sup>a</sup>	7.57 ± 5.94 <sup>a</sup>	18.83 ± 2.27 <sup>a</sup>
	0.25	7.70 ± 2.70 <sup>a</sup>	0.00 ± 2.30 <sup>a</sup>	0.13 ± 3.60 <sup>a</sup>	23.98 ± 2.78 <sup>a</sup>
	0.5	10.07 ± 3.51 <sup>a</sup>	5.45 ± 0.29 <sup>a</sup>	3.62 ± 7.23 <sup>a</sup>	14.20 ± 2.41 <sup>a</sup>
	1	5.06 ± 0.06 <sup>a</sup>	5.28 ± 3.06 <sup>a</sup>	6.32 ± 5.19 <sup>a</sup>	26.03 ± 4.70 <sup>a</sup>
Control	0	2.50 ± 1.34	7.50 ± 2.67	7.50 ± 2.50	40.99 ± 2.07 <sup>b</sup>

There is no significant difference between the values in a column followed by the same letter ( $p < 0.05$ ).

**Table 4.** Contact lethal dose of *P. crispum* and *P. racemosa* essential oils that kills 50% of exposed *D. porcellus*.

Essential oil	Exposure time (h)	LD <sub>50</sub> (µL/adult)	Slope (± S.E.)	Intercept	χ <sup>2</sup>	P-value
<i>P. crispum</i>	24	440.23	0.407 ± 0.66	3.923	7.50	0.10
	48	2.32	0.191 ± 0.26	4.703	9.21	0.83
	72	1.15	0.051 ± 1.03	4.988	1.58	0.66
<i>P. racemosa</i>	24	63.33	0.415 ± 0.97	4.252	9.72	0.98
	48	196.57	0.457 ± 0.58	3.951	1.00	0.00
	72	414.38	0.459 ± 0.63	3.788	8.99	0.02

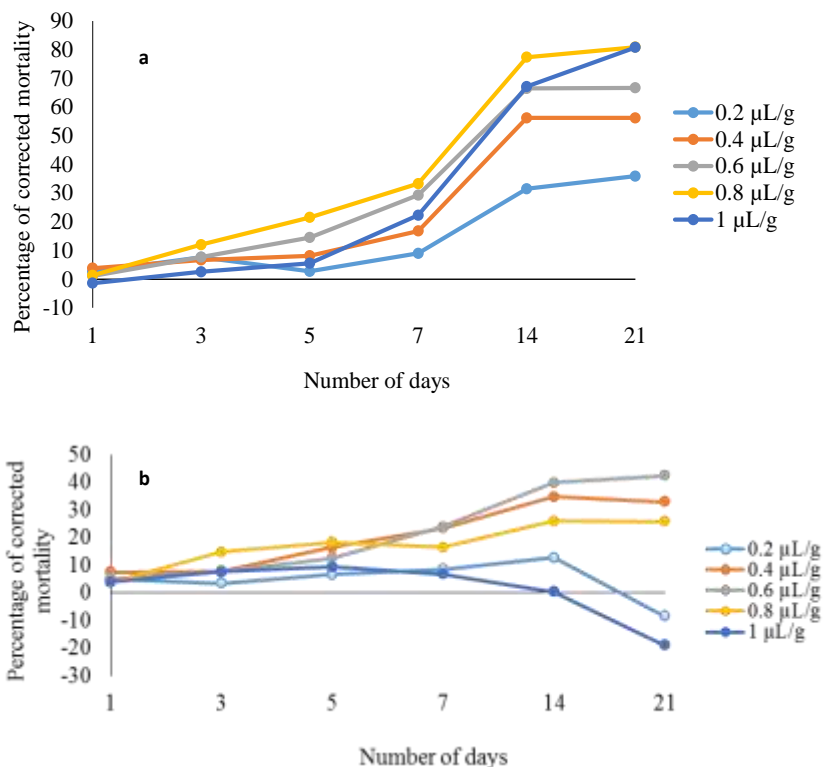
CI: Confidence interval, S.E.: Standard error; χ<sup>2</sup>: Chi-square.

significant ( $P \geq 0.05$ ). The mortality of *D. porcellus* did not vary significantly ( $P \geq 0.05$ ) according to the diverse concentrations of both essential oils tested at 24, 48 and 72 h of exposure time. After 72 h of exposure, *P. crispum* essential oil at 0.1% of concentration caused the higher mortality (21.30 ± 1.87%) of *D. porcellus* (Table 3). While, only after 24 h of exposure, *P. racemosa* essential oil at 0.5% of concentration caused the higher mortality (10.07 ± 3.51) of *D. porcellus*. At 24 h after exposure, *P. racemosa* essential oil was more toxic (LD<sub>50</sub> = 63.33 µL/adult) than *P. crispum* oil (LD<sub>50</sub> = 440.23 µL/adult) (Table 4). However, at 48 and 72 h interval time, a contrary trend was observed. The topical application of essential oils of *P. crispum* and *P. racemosa* at different concentrations significantly decreased consumption of yam chips ( $F = 7.125$ ;  $df = 8$ ;  $P \leq 0.001$ ) by *D. porcellus* (Table 3).

#### Ingestion toxicity and feeding deterrent activity

The ingestion of treated yam chips with both essential

oils at all concentrations caused a great mortality of *D. porcellus* (Figure 1). The effect of oil concentration of both plants ( $P \leq 0.05$ ) and exposure time ( $P < 0.001$ ) were significant on *D. porcellus* mortality. However, their interaction was not significant ( $P \geq 0.05$ ). The essential oil of *P. crispum* at concentration above 0.2 µL/g, caused high mortalities of *D. porcellus* population with concentrations 0.8 and 1 µL/g causing a corrected mortality rate of 80.82% (Figure 1a). On the other hand, the results obtained with *P. racemosa* essential oil showed that the mortality induced by the highest concentration (1 µL/g) and the lowest concentration (0.2 µL/g) fell respectively in the 7 and 14th experimental day, while mortality increased progressively with mean concentrations (0.4, 0.6, and 0.8 µL/g) (Figure 1b). At 1 day interval time *P. racemosa* essential oil (LC<sub>50</sub> = 0.001 µL/g) caused the higher feeding toxicity of *D. porcellus* than *P. crispum* oil (LC<sub>50</sub> = 0.002 µL/g). However, at 21 days interval time, a contrary trend was observed (Table 5). The two essential oils significantly protected ( $F = 2.680$ ,  $df = 45$ ,  $P \leq 0.05$ ) the yam chips compared to the control (Figure 2). Essential oil of *P. crispum* at 4 µL/mL



**Figure 1.** Ingestion toxicity of (a) *P. crispum* and (b) *P. racemosa* essential oils on *D. porcellus*.

**Table 5.** Toxicity by ingestion of *P. crispum* and *P. racemosa* essential oils on *D. porcellus*.

Essential oil	Exposure time (days)	LC <sub>50</sub> (µL/g)	Slope (± S.E.)	Intercept	$\chi^2$	P-value
<i>P. crispum</i>	1	0.002	-0.574 ± 0.73	3.315	4.38	0.077
	3	18.35	3.388 ± 0.08	0.719	2.73	0.434
	5	178.81	1.051 ± 0.33	2.364	2.02	0.000
	7	312.50	0.746 ± 0.33	3.131	9.21	0.957
	14	33.10	0.753 ± 0.25	3.857	8.84	0.901
	21	3.88	2.325 ± 0.42	1.261	1.30	0.729
<i>P. racemosa</i>	1	0.001	-0.286 ± 1.05	3.547	3.20	0.792
	3	218.79	1.027 ± 0.33	2.408	1.06	0.001
	5	2.96	1.982 ± 0.25	3.952	1.56	0.012
	7	31.28	1.869 ± 0.13	2.205	9.98	0.000
	14	1.41×10 <sup>5</sup>	0.338 ± 0.84	3.182	3.60	0.002
	21	1.89×10 <sup>61</sup>	-0.030 ± 11.85	3.116	0.01	0.032

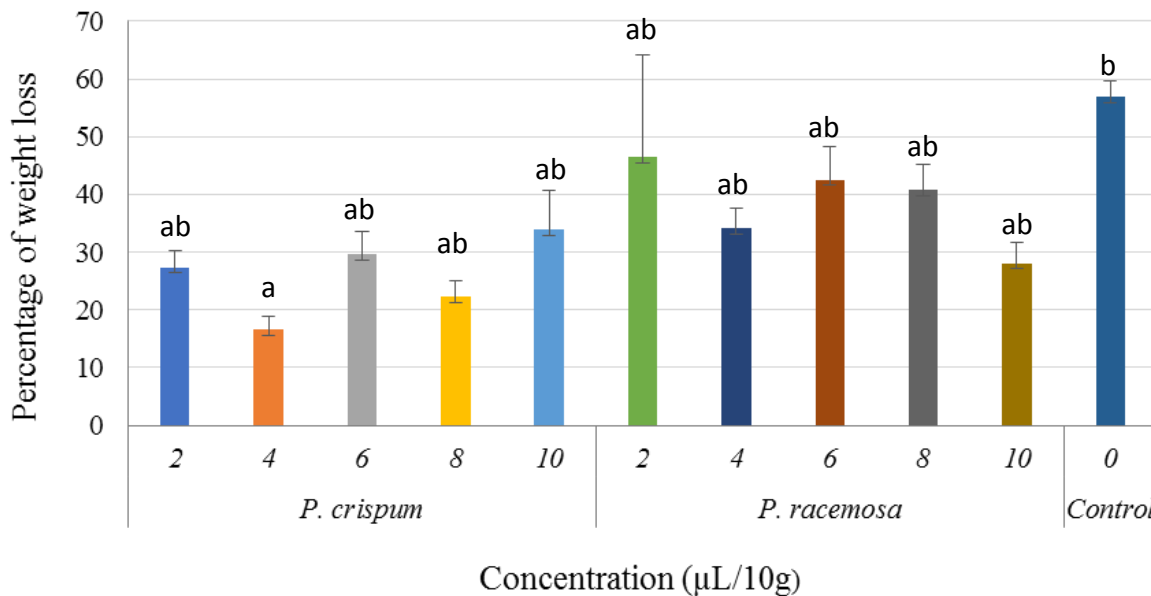
weight loss (Figure 3).

### Fumigant toxicity

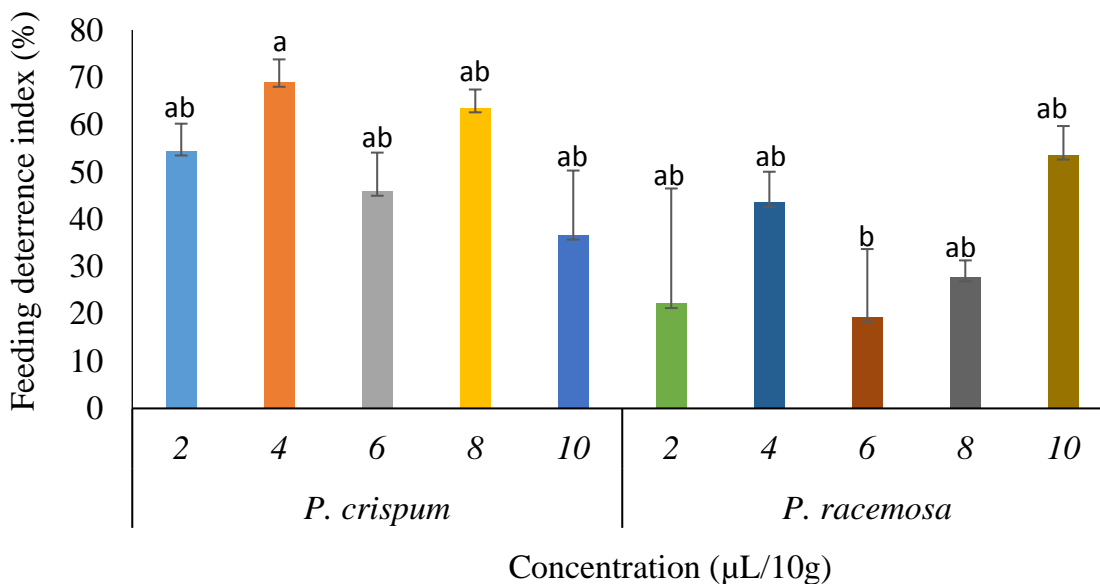
The fumigant activity of *P. crispum* essential oil on *D. porcellus* was significantly ( $P \leq 0.001$ ) higher than those

of *P. racemosa* oil. The increase of oil concentration of both plants and exposure time had a significant ( $P \leq 0.05$ ) effect on the mortality of *D. porcellus*. After 7 days of exposure time, the mortality of *D. porcellus* reached 47.32% when exposed to *P. crispum* essential oil at 20 µL/L air (Figure 4). The essential oil of *P. racemosa* at all concentrations and various periods showed weak





**Figure 2.** Weight loss caused by *D. porcellus* on yam chips treated with *P. crispum* and *P. racemosa* essential oils at 21 days after exposure.

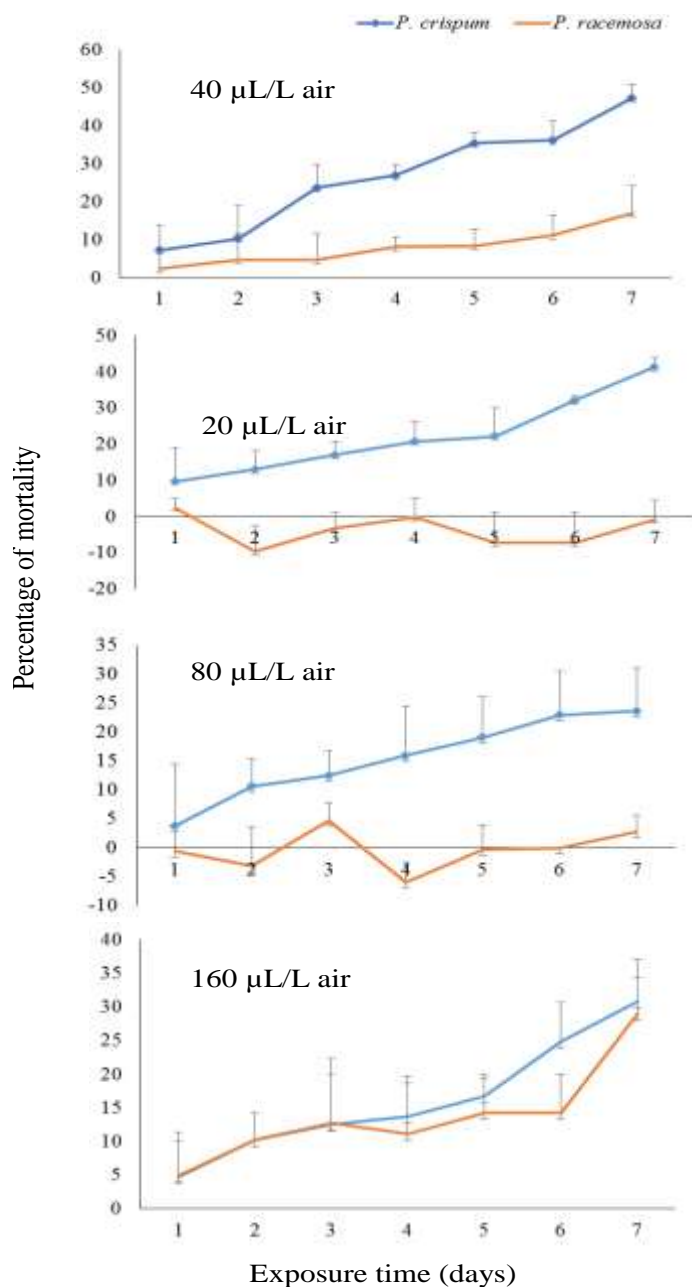


**Figure 3.** Feeding deterrence indices of treated yam chips with *P. crispum* and *P. racemosa* essential oils on *D. porcellus* after 21 days of exposure.

fumigant toxicity with higher mortality of *D. porcellus* (28.96%) at the higher oil concentration (160 µL/L air) 7 days after exposure. *P. crispum* essential oil had very lower LC<sub>50</sub> values than those of *P. racemosa* (Table 6). *D. porcellus* adults fumigated with 160 µL/L air concentration of the *P. crispum* essential oil caused significant less damage (F = 1.94; df = 8; P ≤ 0.05) to yam chips than control at 7 days interval time (Figure 5).

## DISCUSSION

The yields obtained in *P. racemosa* and *P. crispum* leaves essential oils are similar to those obtained by Alitonou et al. (2012), Craft and Setzer (2017), which are in the order of 0.9 to 2.4% and 0.2 to 0.6%, respectively. Alitonou et al. (2012) revealed that chemical composition of *P. racemosa* leaves essential oils from Benin contains



**Figure 4.** Mortality of *D. porcellus* caused by *P. crispum* and *P. racemosa* essential oil fumigants.

24 compounds with eugenol and myrcene as the main important. While, composition of *P. crispum* leaves essential oil cultivated in Africa was revealed by some studies with myristicin and apiol as the main compounds (Nawel et al., 2014; Snoussi et al., 2016; Agyare et al., 2017).

The investigation of bioactivities of *P. crispum* and *P. racemosa* essential oils against *D. porcellus* showed their repellent activity. The repellent activity of *P. crispum* and *P. racemosa* oils could be due to the presence of major

active compounds such as myristicin and eugenol, respectively. Indeed, You et al. (2015) have demonstrated that myristicin and eugenol have strong repellent effect respectively to the stored product insects *Liposcelis bostrychophila* Badonnel and *C. maculatus*. Moreover, myristicin and eugenol were strongly repellent against *Lasioderma serricorne* (Fabricius) (Du et al., 2014) and *Dinoderus bifloveatus* Wollaston (Ojimekwe and Adler, 2000) adults, respectively. However, in this study both tested essential oil showed lower repellency class against *D. porcellus*. Karahroodi et al. (2009) found similar results and showed that *P. crispum* had weak repellency on adults of *P. interpunctella*. While, it has been reported that *P. crispum* had strong repellency activity against *Trialetrodes vaporariorum* (Westw.) in greenhouse tomato production (Tasli et al., 2017). Knowing that the repellent activity of essential oils quickly decrease due to their low molecular masses and high volatility (Jannatan and Rahayu, 2021), this could be explained by the decrease of repellent effect of lower concentration *P. crispum* oil with time.

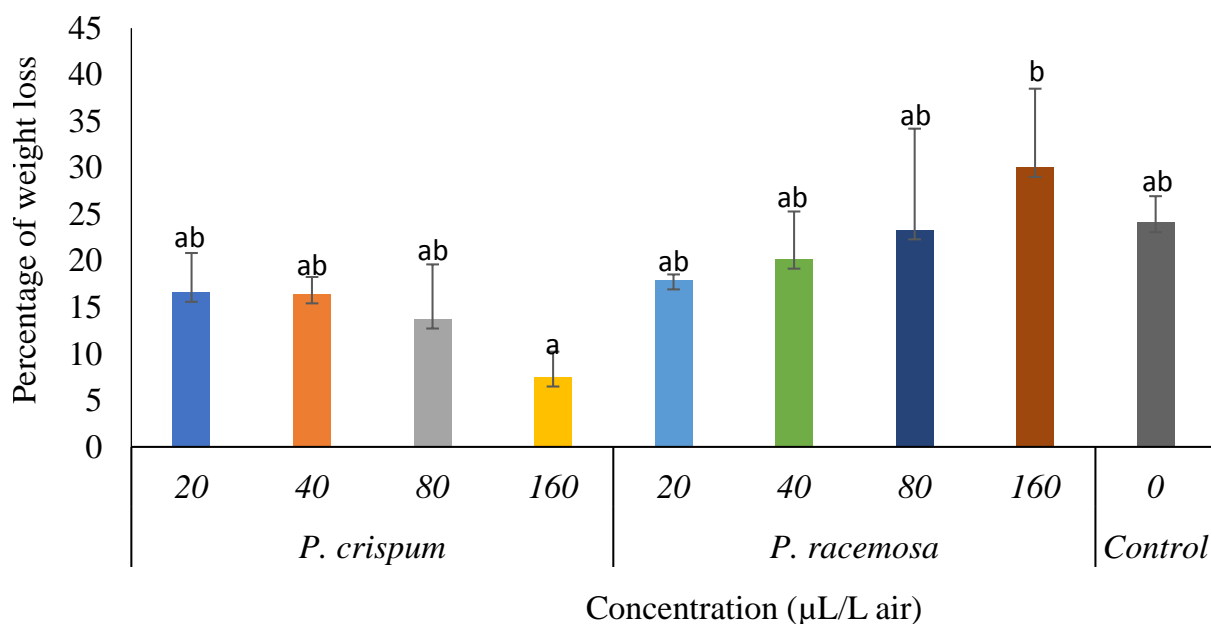
The decrease of the repellent activity of *P. racemosa* essential oil with increase of concentration could be explained by the interaction and/or inactivation of some oil components at high concentration. In fact, when eugenol the major bioactive compound of *P. racemosa* essential oil was methylated, methyl-eugenol, which can be contained at over 90% in this oil (Tan and Nishida, 2012), may play a physiological role on the toxicity reduction (Chang et al., 2009). The understanding of interaction among compounds contained in *P. racemosa* but also in *P. crispum* essential oils should be an important topic for further research. In general, these results suggest that the essential oils of both plant leaves cannot be used as repellents in the *D. porcellus* control.

The results showed that, *P. racemosa* and *P. crispum* essential oils had insecticidal activity on *D. porcellus* by topical application. Similar findings have been documented by Leyva et al. (2007a, b) which showed that *P. racemosa* essential oil had higher toxicity against *Musca domestica* L. and *Blatella germanica* L., respectively. Moreover, *P. racemosa* oil caused total mortality of *Spodoptera littoralis* (Boisduval) higher than 70% (Pavela, 2013). The toxic effect of *P. racemosa* essential oil to *D. porcellus* could be attributed to the presence of eugenol. In fact, eugenol applied topically on *Sitophilus granarius*, *Sitophilus zeamais*, *Tribolium castaneum* and *P. truncatus* was highly toxic (Obeng-Ofori and Reichmuth, 1997; Huang et al., 2002). Contrary to *P. crispum* essential oil, the contact toxicity of *P. racemosa* oil did not persist with time. The lower persistence of eugenol (Obeng-Ofori and Reichmuth, 1997) could explain these phenomena. Indeed, the loss of toxicity of *P. racemosa* essential oil with exposure time could be due to their high volatility and the quick degradation of active compounds. Concerning *P. crispum* essential oil, its insecticidal activity has been reported



**Table 6.** Lethal concentration of *P. crispum* and *P. racemosa* essential oil fumigants that kills 50% of exposed *D. porcellus*.

Plant	Exposure (days)	LC <sub>50</sub> (µL/L air)	Slope (± S.E.)	Intercept	χ <sup>2</sup>	P-value
<i>P. crispum</i>	1	0.000	-0.552 ± 5.48	3.692	0.67	0.907
	2	0.000	-0.094 ± 2.82	3.912	0.15	0.274
	3	0.004	0.215 ± 1.07	4.460	3.48	0.789
	4	0.078	-0.323 ± 0.72	4.639	8.69	0.958
	5	2.479	-0.535 ± 0.41	5.221	9.69	0.969
	6	2.678	-0.431 ± 0.49	5.185	8.79	0.935
	7	7.224	-0.452 ± 0.45	5.388	6.86	0.804
<i>P. racemosa</i>	1	1.32×10 <sup>5</sup>	0.519 ± 0.72	2.261	6.00	0.758
	2	2.28×10 <sup>12</sup>	0.120 ± 2.86	3.102	0.44	0.108
	3	3382.68	0.877 ± 0.38	1.768	5.71	0.074
	4	5775.21	0.773 ± 0.40	2.092	1.00	0.999
	5	2.05×10 <sup>9</sup>	0.072 ± 4.08	3.445	0.05	0.010
	6	5.07×10 <sup>8</sup>	0.183 ± 1.47	3.411	1.02	0.997
	7	3.39×10 <sup>6</sup>	0.134 ± 1.81	3.718	0.17	0.002

**Figure 5.** Weight loss of yam chips caused by *D. porcellus* fumigated with essential oils of *P. crispum* and *P. racemosa* after 7 days of exposure.

against *Aedes aegypti* L. (Intirach et al., 2016). The insecticidal activities of *P. crispum* essential oil can be attributed to the presence of monoterpenoids such as myristicin, which can penetrate into insects and affect their physiological functions (Ebadollahi, 2011). Indeed, the compounds of essential oils exert their activities on insects through neurotoxic effects (Regnault-Roger et al., 2012). Świech and Poleć (2013) have reported that myristicin from *P. crispum* essential oil

showed an insecticidal activity against housefly (*M. domestica* L.) and oriental cockroach (*Blatta orientalis* L.). Moreover, high contact toxicity of myristicin against *L. serricornis* adults has been found by Du et al. (2014). Despite the low toxicity of both essential oils, the topical application of these oils on *D. porcellus* had significantly decreased yam chips weight loss showing their potential role in management of this pest.

In this study, *P. crispum* and *P. racemosa* essential oils

demonstrated high insecticidal activity against *D. porcellus* via ingestion of treated yam chips. These results are in accordance with Noudogbessi et al. (2008) which showed high toxicity of treated maize with *P. racemosa* essential oil against *P. truncatus*. The toxicity of both essential oil could be due to the penetration of essential oil compounds in the insect body through the digestive system. These essential oil compounds could act by increasing the gut pH or a reduction in  $\alpha$ -amylase activity, which lead to insect mortality (Stefanazzi et al., 2011). Moreover, in treated yam chips, apart from the ingestion toxicity, *D. porcellus* could be rapidly coated with essential oil compounds which lead to contact toxicity by penetration into insect cuticle. The results of this study showed that at 21 days interval time, the mortality of *D. porcellus* due to *P. racemosa* essential oil was absent for the lowest and highest tested concentrations. To understand this phenomenon, on the one hand, the identification of the bioactive compounds in the *P. racemosa* essential oil against *D. porcellus* is necessary and on the other hand the interaction effect of these bioactive compounds must be investigated.

This study revealed that the essential oil of *P. crispum* and *P. racemosa* act as feeding deterrents against *D. porcellus*. Similar investigations were made by Sharaby and El-Nojiban (2015) who showed the feeding deterrence activity of *P. crispum* on *Agrotis ipsilon* (Hufnagel) larvae. Moreover, similarly to this study, Sharaby and El-Nojiban (2015) demonstrated that *P. crispum* essential oil caused high feeding deterrence against *A. ipsilon* but a low contact mortality. The ability of essential oils to reduce feeding of *D. porcellus* is probably due to the presence of main compounds myristicin and eugenol in *P. crispum* and *P. racemosa* essential oils, respectively. Indeed, myristicin exhibited strong antifeedant effect on larvae and imagoes of *Brontispa longissima* (Gestro) (Qin et al., 2010). Similarly, eugenol from *Laurus nobilis* L. essential oil had a feeding deterrent activity against *Mythimna unipuncta* (Haworth) (Muckensturm et al., 1982). Knowing that, oil compounds reduce feeding activity of *D. porcellus* acting on gustatory chemoreceptors (Akhtar et al., 2012), it is important to identify the role of each oil compound to the feeding deterrent effect of both tested oils.

The essential oil of both plants exhibited fumigant activities against *D. porcellus* with different efficacies. According to Jannatan and Rahayu (2021) essential oil fumigants enter the insect body through the tracheae. The fumigant toxicity of *P. crispum* essential oil against *D. porcellus* was dose-dependent and higher than those of *P. racemosa*. This is not surprising because it is known from previous works that *P. crispum* essential oils have strong fumigant toxicity on some storage insect pests such as *C. maculatus* (Massango et al., 2017; Maroufpoor et al., 2016), *Ephestia kuehniella* Zeller and *Plodia interpunctella* (Hübner) (Maroufpoor et al., 2016), but also on field crop insects such as *Trialeurodes vaporariorum* (Westwood) (Mahmoodi et al., 2014). The

fumigant toxic activity of *P. crispum* essential oil could be related to the presence of myristicin. Indeed, the fumigant toxicity of myristicin contained in *P. crispum* essential oil had been shown against *T. vaporariorum* (Mahmoodi et al., 2014). The insecticidal activity of *P. racemosa* essential oils inhaled by *D. porcellus* was weak and more or less stable. Furthermore, contrasting results were reported by Leyva et al. (2007a) and Lee et al. (2002), which showed that, *P. racemosa* had high fumigant toxicity against *M. domestica* and *T. castaneum*, respectively. These discrepancies in biological activity of *P. racemosa* could be related to the differences of chemical composition and tested insect species. The fumigant toxicity of *P. racemosa* could be attributed to the presence of eugenol as major constituent. Indeed, eugenol has been shown to exhibit a fumigant property toward *C. maculatus* (Ajayi et al., 2014). The results showed that the fumigation of *D. porcellus* had significantly reduced the yam chips weight loss. The reduction in food intake by fumigated *D. porcellus* in comparison with control gave a clear picture of the efficacy of both essential oils used as fumigant (Jaya et al., 2012). Fumigant activity of *P. crispum* essential oil is quite promising and they show potential to be developed as possible natural fumigants for control of stored product insects.

## Conclusion

In this paper, we report repellent, contact, feeding deterrence, and fumigant activities of essential oils of *P. crispum* and *P. racemosa* leaves against *D. porcellus* for the first time. Both essential oils showed low repellence against *D. porcellus*. While, when they are in contact with *D. porcellus*, both essential oils not only induced mortality but also significantly reduced yam chips weight loss caused by this pest compared to control. However, the ingestion toxicity of essential oil of *P. crispum* was higher than *P. racemosa*. Moreover, *P. crispum* essential oil showed feeding deterrence and fumigant activity against *D. porcellus* adults showing its potential to be developed into antifeedant and fumigants to control against this pest. However, further investigations are necessary to determine which compounds contributing to the toxicity of both essential oils against *D. porcellus*.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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