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# Repellency and insecticidal properties of seed oil of *Jatropha curcas* L. against American cockroach, *Periplaneta americana* L.



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### **Abstract**

**Background:** This study evaluated the effect of *Jatropha curcas* seed oil against adult American cockroach, *Periplaneta americana*, a mechanical disease vector, using three bioassay methods to determine the repellent activity, contact and fumigant toxicity. This involved the use of *J. curcas* oil solution (diluted with acetone (20%)) and *J. curcas* pure oil. For repellency test, concentrations 0.30, 0.60 and 0.90% v/v were used for the oil solution while 1.0 and 2.0 ml concentrations were used for the pure oil. All test groups were exposed for 15 min. Contact toxicity test involved the use of 0.30, 0.60, 0.90, 1.20 and 1.50% v/v concentrations for the oil solution while 1 and 2 ml concentrations were used for the pure oil. Exposure period for all test groups was 24–120 h. For the fumigant test, 0.15% v/v and 0.5 ml concentrations were used for the oil solution and pure oil groups respectively; exposure period for the test groups was 24–120 h. All test and the control groups had ten cockroaches (*P. americana*) per group with four replicates.

**Results:** Repellency was higher in test groups treated with pure *J. curcas* oil than in groups treated with the oil solution with repellency of 70–100% and 60–100% respectively after 15 min exposure period. For the contact test, a higher mortality rate was observed with the oil solution than the pure oil. Mortality was lower for 1 ml of pure oil with 20% at 24 h and 40% at 120 h than 2 ml of pure oil with 30% mortality at 24 h and 50% mortality at 120 h. A 100% mortality was recorded in the highest concentration (1.50% v/v) at 120 h. Fumigation test with 0.15% v/v of oil solution resulted in 20% mortality at 120 h while fumigation test with 0.5 ml of *J. curcas* pure oil resulted in 60% mortality at 120 h.

**Conclusion:** *J. curcas* seed oil possesses repellent and insecticidal properties against *P. americana*. Thus, the menace caused by this mechanical disease vector could be reduced using *J. curcas* seed oil.

Keywords: Repellency, Insecticidal, Fumigant, Jatropha curcas, Periplaneta americana

### Introduction

Cockroaches are insects of the order Blattodea; they are the most abundant and obnoxious non-biting insect pests in residential buildings, hospitals, hotels and restaurants (Bala & Sule, 2012; Kumar & Tewari, 2015).

Cockroaches are abundant throughout the world and live in a wide range of environments especially in the tropics and subtropics (Bell, Roth, & Nalepa, 2008). Many species of cockroaches live in leaf litters, rotting wood, holes, stumps, cavities, tree barks, log piles and debris (Bell et al., 2008). There are about 4600 recognized cockroach species, of which about 30 species are associated with human habitat (Dingha, O'Neal, Appel, & Jackai, 2016). Only about four widespread species are

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commonly regarded as pests (Schal & Hamilton, 1990; Valles, Koehler, & Brenner, 1999). The four species include *Periplaneta americana*, *Blatella germanica*, *Blatta orientalis* and *Supella longipalpa* (Nalyanya, Moore, & Schal, 2000; Sulaiman et al., 2011). In Nigeria, *P. americana* and *B. germanica* are the two most common and notorious cosmopolitan pest species (Anikwe et al., 2014; Hamu et al., 2014; Nazari, Motlagh, & Nasirian, 2016).

Cockroaches may contaminate food and eating utensils, destroy fabric and paper products and impart stains and unpleasant odour to the surfaces they come in contact with (Rejitha, Reshma, & Mathew, 2014). Cockroaches not only contaminate food with their droppings but they also cause food poisoning (Moges et al., 2016). According to Tatfeng, Usuanlele, and Orukpe (2005), antigens and faeces of cockroaches result in asthmarelated health problems. Cockroaches are of great public health importance as they can serve as hosts, reservoirs and mechanical carriers of pathogenic organisms such as bacteria (Tatfeng et al., 2005), parasites and viruses (Bala & Sule, 2012; Etim, Okon, Akpan, Ukpong, & Oku, 2013; Fotedar, Nayar, & Samantray, 1989; Nagham, Anfal, & Israa, 2011; Salehzadeh, Tavacol, & Mahjub, 2007). Cockroaches feed readily on faeces, sputum, skin scrapings and other human detritus as well as a variety of food stuffs. These filthy behaviour of cockroaches makes them ideal vectors of a wide range of pathogens such as Escherichia coli, Pseudomonas aeruginosa, Klebsiella sp., Shigella dysenteriae, Bacillus cereus and Entamoeba histolytica, Ascaris lumbricoides, Trichuris trichiura, hookworms, Enterobius vermicularis, Hymenolepis nana, Toxocara canis and larvae of Strongyloides stercoralis (Baumholtz, Parish, Witkowski, & Nutting, 1997; Moges et al., 2016; Tachbele, Erku, Gebre-Michael, & Ashenafi, 2006; Tatang, Tsila, & Pone, 2017).

The damages caused by cockroaches are enormous and difficult to estimate ranging from inconvenience to disease transmission. Therefore, there is need to reduce their number below injury level. Control approaches involve the use of chemical insecticides such as pyrethroids, carbamates, organochlorines, organophosphates, hydramethylnon and sulfluramid. These insecticides have undesirable effects on the environment due to their hazard and are known to pose public health issues (Khan, Qamar, Mehdi, & Shahid, 2011; Tunaz, Er, & Isikber, 2009). Therefore, the need to introduce biodegradable insecticides that will help reduce the population of this group of insects is desirable.

Jatropha curcas belongs to the family Euphorbiaceae and subfamily Acalyphoideae. It is a multipurpose perennial shrub, native to Mexico and subtropical America and found in most tropical areas of the world including Nigeria (Gübitz, Mittelbach, & Trabi, 1999; Janick &

Paull, 2008). Series of studies revealed that J. curcas possesses some insecticidal and larvicidal properties (Ahirrao et al., 2011; Rahuman, Gopalakrishnan, Venkatesan, & Geetha, 2008; Silva, Faroni, Sousa, & Freitas, 2012). Almost all parts of J. curcas are useful. The seeds of J. curcas consist of saponins, lectins (curcin), phytates, protease inhibitors, curcalonic acid and phorbol esters (Makhar, Francis, & Beckar, 2007). Isman (2006) reported that plant extracts and oils have wide range of activities against pests, including insect vectors. These natural products have fumigant, antifeedant and repellent effects as well as inhibitory effect on their reproduction (Omara, Al-Ghamdi, Mahmoud, & Sharawi, 2013). This study was carried out to investigate the repellency effect and insecticidal properties of seed oil of J. curcas on the American cockroach, P. americana.

### Materials and methods

### Collection and identification of J. curcas

J. curcas seeds (Fig. 1a) used were collected from Mkpaha Ibok in Aka Offot, Uyo Local Government Area, Akwa Ibom State, in April, 2019. The plant was identified at the herbarium of the Department of Botany and Ecological Studies, Faculty of Science, University of Uyo, Akwa Ibom State, Nigeria, with herbarium number UUH 4033 (Uyo).

### Oil extraction from seeds of J. curcas

The extraction of oil from the seeds of *J. curcas* was done at the laboratory of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Akwa Ibom State, Nigeria. Seeds collected were de-shelled, washed with clean water, dried under room temperature and pulverized using a crusher machine. The pulverized seeds (Fig. 1b) which weighed 697 g was poured into a glass jar and labeled.

The methods of Akbar, Siddiqui, Sagathevan, and Khan (2019) and Shivani, Khushbu, Faldu, Thakkar, and Shubramanian (2011) were adopted in the extraction process. A quantity of 2.5 l of n-Hexane was used for the extraction. The first half of n-Hexane (1.25 l) was poured into a glass jar containing the pulverized seeds. The jar was periodically shaken, and after 72 h, the mixture was filtered using Whatman No. 1 filter paper, and this was followed by the evaporation of n-Hexane from *J. curcas* oil using a rotary evaporator. This was done *in vacuum* and at a temperature of 40 °C. *Jatropha* seed oil was stored in an air-tight container and maintained at room temperature until it was needed for the experiment.

### Collection and identification of P. americana

Two methods were used for the collection of adults of *P. americana* which included the use of baited traps and



hand collection method. The bait was prepared according to the method used by Abdel-Gahny, Zalat, Abo-Ghalia, and Semida (2008) where the bait consisted of attractants such as fresh fruits, sugar and wet bread. Also, cockroaches were collected by hand picking them using sterile hand gloves, around toilets, bathrooms and septic tanks. The collection was done at Itu road, Uyo (latitude 5° 2′ 25.77″ N and Longitude 7° 58′ 44.44″ E) in October, 2019. Cockroaches of the species *P. americana* collected were identified using standard taxonomic, morphological and pictorial keys as described by Ross (1965). *P. americana* is reddish brown to brown in colour with light yellow bands around the shield behind the head that is there is substantial variation in light and dark patterns on the pronotum.

Adults of *P. americana* collected were placed in  $24 \times 40\,\mathrm{cm}$  plastic containers and maintained at a temperature of  $30 \pm 2\,^{\circ}\mathrm{C}$  and photoperiod of  $12:12\,\mathrm{(L:D)}$  hour, in the laboratory of the Department of Animal and Environmental Biology, Faculty of Science, University of Uyo, Nigeria. The four walls of the containers were smeared with a thin layer of vaseline (blue seal) as described by Thavara et al. (2007) to prevent escape of cockroaches, and they were fed with dry crumbled biscuits, bread and water. Pieces of facial tissue were provided as harborage. They were allowed  $48\,\mathrm{h}$  to get used to the laboratory environment before commencement of the experiments.

### **Experimental design**

A stock solution of the oil of *J. curcas* was prepared using 20% acetone as the diluent. From the stock solution, five different concentrations of the oil were obtained: 0.30, 0.60, 0.90, 1.20 and 1.50% v/v. Four replicates were used for each test concentration. A control experiment was also set up; this consisted of 3 ml acetone (20%). The experiments determined the repellency, contact and fumigant toxicities of *J. curcas* on adults of *P. americana*.

In this study, the repellency, contact and fumigant toxicities of pure (100%) *J. curcas* oil were also determined; this consisted of 1 and 2 ml of the pure (100%) oil of *J. curcas*. The control for this set up consisted of 3-ml distilled water.

### Repellency test

The methods of Appel, Gehret, and Tanley (2001), Manzoor, Munir, Amdreen, and Naz (2012) and Sharififard, Safdari, Siahpoush, and Kassiri (2016) were adopted for this study. Whatman No. 1 filter paper was divided into two equal pieces. In each case, one half of the filter paper was treated with the test oil solution, using a micro sampler, and the other half was left untreated. Different concentrations of the test oil solution (0.30, 0.60, 0.90% v/v) and their replicates were used for the treatment. Each treated filter paper was left for 2 min to dry before commencement of the repellency test. This was followed by placing the filter paper at the bottom of rectangular assay plates (on the right side). The untreated paper was placed on the left side of the assay plate.

Ten (10) adults of both sexes of *P. americana* were released into the centre of each assay plate. The distribution of the test insects was observed at 0, 5, 10, and 15 min. After this exposure period, repellency was calculated using the formula shown below.

Repellency (%) = 
$$100 - \frac{T \times 100}{N}$$

where

T = number of cockroaches found in the treated area N = the total number of cockroaches used.

The repellency effect of pure (100%) *J. curcas* oil was also determined. This consisted of 1 and 2 ml of the pure (100%) oil of *J. curcas*. The control for this set up

consisted of 3-ml distilled water. Exposure period was also 0, 5, 10 and 15 min.

### Contact toxicity

The bioassay method of the WHO (1975) was used to determine the susceptibility of cockroaches to the fixed oil of *J. curcas*. Whatman No. 1 filter papers (whole) were separately treated with 0.30, 0.60, 0.90, 1.20 and 1.50% v/v of *J. curcas* oil. Each of these concentrations had four replicates. Each filter paper was first left to dry for 2 min and then was folded into a 500-ml capacity jar. The top inner surfaces of these assay jars were smeared with a thin layer of Vaseline (blue seal) to prevent the escape of cockroaches from the jars.

Ten (10) adult cockroaches of both sexes were introduced into each of the jars and observed for mortality for 24, 48, 72, 96 and 120 h after treatment as described by Thavara et al. (2007) and Zibaee, Khorram, and Hamoni (2016). A control experiment was set up with four replicates also. Cockroaches in the control groups were exposed to filter papers treated with 3 ml acetone (20%).

Contact toxicity was also determined using pure (100%) *J. curcas* oil. Two volumes (1 and 2 ml) of the oil were used for this test. Ten cockroaches each were used in this test, with four replicates. For the control experiment, cockroaches (10) were exposed to filter paper treated with 3-ml distilled water. Exposure period was the same as for the cockroaches exposed to the oil solution.

### **Fumigant toxicity**

Fumigant toxicity tests reported in this study were carried out as described by Appel et al. (2001), Thavara et al. (2007) and Zibaee et al. (2016). One-centimeter (1 cm) diameter cotton ball was treated with 0.15% v/v of *J. curcas* oil. To prevent direct contact of cockroaches with the fixed oil, it was injected to the centre of each cotton ball using a micro sampler. This was replicated four times. Each cotton ball was separately introduced into 1-l capacity test jar.

The control groups which also had four replicates were exposed to cotton balls treated with 0.5 ml acetone (20%). Observation for mortality and other toxic effects of the oil on the test insects was made at 24, 48, 72, 96 and 120 h, for both the test and control experiments. A quantity of 0.5 ml of pure *Jatropha* oil (100%) was also used for fumigant toxicity tests where 0.5 ml of distilled water served as the control. Exposure time was also 24, 48, 72, 96 and 120 h. Each experimental set up had 10 cockroaches of both sexes.

### Data analysis

One way analysis of variance (ANOVA) was used to determine the level of significance among the concentrations used in the various groups. Analysis was performed using statistical package for social sciences (SPSS) version 21.0.

### Results

### Repellent effect of J. curcas oil on P. americana

The repellent effect of pure oil and oil solution of J. curcas is shown in Table 1. The study revealed that there was increasing repellent effect on P. americana at higher concentrations. For the oil solution, the highest repellency was observed at 0.90% v/v from 0 to 15 min. Also, it was observed that concentrations of 0.60% and 0.90% v/v both had 100% repellent effect on P. americana at the start of the experiment. The 0.30% v/v had the least repellent effect on P. americana with a percentage repellency of 60% at 15 min of exposure, although the repellent effect was as high as 90% (Table 1), at the start of the experiment. Reduction in repellency was observed for all concentrations with increase in exposure time. Analysis of variance revealed that there was no significant variation (p = 0.112) in the repellent effect of different concentrations of J. curcas oil on the test cockroaches. Although, the repellency was high for all the groups, acetone (the control) was observed to have a higher repellent effect on P. americana than the concentrations of J. curcas tested in this study and its effect did not appear to be time dependent.

For the effect of pure oil on *P. americana*, 1 ml and 2 ml of pure *J. curcas* oil performed well with no significant repellent effect between the two concentrations. One millilitre concentration had 70% repellent effect at the beginning of the study while 2 ml concentration had 80% repellent effect. There was no significant difference (p > 0.05) in the repellent effect between the two. It was observed that the pure oil had increasing repellency effect. The repellency increased with increase in exposure time (Table 1).

### Contact toxicity effect of J. curcas oil on P. americana

Mortality of *P. americana* exposed to *J. curcas* oil solution increased with increase in exposure time and oil concentration. A 100% mortality was observed for 1.20% v/v concentration at 96 and 120 h and for 1.50% v/v concentration at 72-, 96- and 120-h exposure period. No death was observed for 0.30% v/v concentration at 24- and 48-h exposure period. For the control experiment, no mortality was observed at 24 h, whereas mortality of 80% was observed at 120 h. When *P. americana* was exposed to the highest concentration of 1.50% v/v of the oil solution at 48 h, a physical change in colour was

**Table 1** Repellent effect of oil solution and pure oil of *J. curcas* on *P. americana* 

Concentration % (v/v)	Total number repelled (%)/time of exposure					
	0 min (n = 40)	5 min ( <i>n</i> = 40)	10 min (n = 40)	15 min (n = 40)	repellence ± SD	
Oil solution						
0.30	36 (90.0)	28 (70.0)	28 (70.0)	24 (60.0)	$29 \pm 5.03^{a}$	
0.60	40 (100.0)	32 (80.0)	28 (70.0)	24 (60.0)	$31 \pm 6.83^{a}$	
0.90	40 (100.0)	36 (90.0)	32 (80.0)	32 (80.0)	$35 \pm 3.83^{a}$	
Pure oil						
1.00	28 (70.0)	32 (80.0)	40 (100.0)	40 (100.0)	$35 \pm 6.00^{a}$	
2.00	32 (80.0)	32 (80.0)	36 (90.0)	40 (100.0)	$35 \pm 3.83^{a}$	
Control (3 ml of 20% acetone)	40 (100.0)	40 (100.0)	36 (90.0)	40 (100.0)	$39 \pm 2.00^{a}$	

n = total number of cockroaches introduced in each group. Means with the same alphabet along the same column are not significant at p > 0.05

observed from the reddish brown colour possessed by *P. americana* to a pale brown colour.

For the pure oil, mortality was observed in both concentrations throughout the exposure time (24, 48, 72, 96 and 120 h). When test organisms were exposed to 1 and 2 ml of pure *J. curcas* oil, 20% and 30% mortality were respectively, after 24-h exposure period. At the highest exposure period (120 h), 40% and 60% mortality were observed for 1 ml and 2 ml pure oil respectively (Table 2).

Comparison of the eight groups revealed that the oil solution at 1.50% v/v had the highest contact effect, followed by 1.20% v/v of oil solution and 2 ml of pure oil of *J. curcas*. These three groups were significantly effective compared to the other groups in their contact effect on *P. americana*.

### Fumigant toxicity effect of J. curcas oil on P. americana

The fumigant toxicity effect of *J. curcas* oil on *P.* americana is presented in Table 3. At 24 h, 5.0% mortality was observed for *J. curcas* oil solution while 30.0% mortality was recorded for groups treated with *J. curcas* pure oil.

At 120 h, 20% mortality was observed for the groups treated with *J. curcas* oil solution, whereas 60% mortality was observed for groups treated with *J. curcas* pure oil. For the control experiment where acetone was used, no mortality was observed at 24- and 48-h exposure period while 20% mortality was observed at 120 h. The control experiment with water gave no mortality throughout the exposure period of 120 h. Analysis of variance revealed that there was significant difference (p < 0.05) in the fumigant toxicity effect between the test oil concentrations and the controls.

### Discussion

This study on evaluation of the efficacy of *J. curcas* oil against *P. americana* is a preliminary investigation. Results obtained revealed the repellent and insecticidal potentials of oils from the seeds of *J. curcas* and corroborate the reports of other researchers. The potency of the oil of *J. curcas* against the maize weevil (*Sitophilus zeamais*), pest of cowpea (*Callosobruchus maculatus*) and the pest of okro (*Podagrica* sp) has been documented by Adebowale and Adedire (2006),

**Table 2** Percentage mortality of *P. americana* on exposure to *J. curcas* pure oil (contact toxicity)

Concentration % (v/v)	Number of dea	Mean				
	24 h (n = 40)	48 h (n = 40)	72 h (n = 40)	96 h (n = 40)	120 h (n = 40)	mortality ± SD
Oil solution						
0.30	0 (0.0)	0 (0.0)	2 (5.0)	4 (10.0)	4 (10.0)	$2.00 \pm 2.00^{d}$
0.60	2 (5.0)	4 (10.0)	8 (20.0)	12 (30.0)	14 (35.0)	$8.00 \pm 5.10^{cd}$
0.90	4 (10.0)	6 (15.0)	12 (30.0)	20 (50.0)	34 (85.0)	15.20 ± 12.21 <sup>bcd</sup>
1.20	8 (20.0)	14 (35.0)	34 (85.0)	40 (100.0)	40 (100.0)	$27.20 \pm 15.14^{ab}$
1.50	14 (35.0)	24 (60.0)	40 (100.0)	40 (100.0)	40 (100.0)	$31.60 \pm 12.03^{a}$
Pure oil						
1.00	8 (20.0)	12 (30.0)	12 (30.0)	16 (40.0)	16 (40.0)	12.80 ± 3.35 <sup>cd</sup>
2.00	12 (30.0)	16 (40.0)	20 (50.0)	20 (50.0)	24 (60.0)	$18.40 \pm 4.56^{abc}$
Control (3 ml 20% acetone)	0 (0.0)	16 (40.0)	16 (40.0)	28 (70.0)	32 (80.0)	18.40 ± 12.52 <sup>abc</sup>

n= total number of cockroaches introduced in each group. Means along the same column with different alphabet(s) are significant at  $p \le 0.05$ 

**Table 3** Fumigant toxicity effect of *J. curcas* oil solution and pure oil on *P. americana* 

Concentration	Number of death (% mortality)/time of exposure						
	24 h (n = 40)	48 h (n = 40)	72 h (n = 40)	96 h (n = 40)	120 h (n = 40)		
0.15% (v/v) of <i>J. curcas</i> oil solution	2 (5.0)	4 (10.0)	4 (10.0)	6 (15.0)	8 (20.0)		
0.5 ml of <i>J. curcas</i> (pure) oil	12 (30.0)	16 (40.0)	20 (50.0)	20 (50.0)	24 (60.0)		
Control with 0.5 ml of 20% acetone	0 (0.0)	0 (0.0)	4 (10.0)	4 (10.0)	8 (20.0)		
Control with 0.5 ml of water	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)		

n = total number of cockroaches introduced in each group

Ohazurike, Omuh, and Emeribe (2003) and Phowichit, Buatippawan, and Bullangpoti (2008), respectively. Addisu, Mohamed, and Waktole (2014) and Yohannes (2006) also tested the effect of *J. curcas* on termites of the species *Microcerotermes beesoni* and *Macrotermes* sprespectively. They reported that the oil had insecticidal effect on these species of termites. Studies on the efficacy of some plant essential oils and powders against *P. americana* have been documented by Rejitha et al. (2014) and Thavara et al. (2007) respectively.

In this study, the pure oil of *J. curcas* performed better than the oil solution in terms of repellency and fumigant toxicity. It was also observed that mortality was higher in the contact toxicity test than in the fumigant test. This may be due to the weak fragrance of the oil. A quantity of 3 ml of 20% acetone which was used in the control experiment demonstrated appreciable repellent effect on the test organisms. However, when acetone (20% acetone) was used in the formulation of the oil solution, it did not appear to improve the performance/potency of the oil. This is an indication that *J. curcas* seed oil with or without diluents holds promising potential as repellent and insecticide against *P. americana*.

The potency of oil from the seeds of *J. curcas* is attributed to its chemical constituents. Adebowale and Adedire (2006), in their studies, detected the presence of sterols and triterpene alcohols in the oil obtained from the seeds of *J. curcas* and commented that these chemical substances have insecticidal properties. Bashir and El Shafie (2013) reported the presence of saponins, lectins (curcin), phytates, protease inhibitors, curcalonic acid and phorbol esters in oil extracted from *J. curcas* seeds. They attributed the insecticidal effect of this oil on the desert locust, *Schistocerca gregaria* to these constituents. Thus, the repellency (60–100%) and mortality observed for the varying oil concentrations tested in this study is also attributable to the chemical compounds present in the oil.

Acda (2009) and Bekele (2002) reported that an active component in the oil of *J. curcas* called jatrophine, a diterpene which is a dominant compound, interfered with the normal message transfer system in the mid gut cell leading to physiological discomfort and death of insects.

The chemical constituents of the seed oil of *J. curcas* may have exerted their effect singly or in synergy. Further investigation is needed to substantiate this. It is thus needful to isolate and characterize the active principle(s) in this test oil.

### **Conclusion**

Oil from the seeds of *J. curcas* possesses repellent and insecticidal properties against *P. americana*. The pure oil proved more effective in repelling the cockroaches than the oil solution. In other words, *J. curcas* seed oil showed a considerable repellent effect on *P. americana*. Appreciable mortality of test cockroaches was observed in both the contact and fumigant toxicity tests. The oil of *J. curcas* holds potential as repellent and insecticide against the American cockroach, *P. americana*.

### Recommendations

Due to the negative impact of the use of chemical insecticides on the environment, it is recommended that ecofriendly insecticides should be used. The observation from this study revealed that the oil of *J. curcas* was effective as repellent and insecticide against adult American cockroach, *P. americana*. *J. curcas* seed oil should be considered for the control of *P. americana* due to its high repellency and insecticidal effects. Better formulations and application methods should be developed to ensure desired results of the oil.

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### Authors' contributions

All authors participated in all parts of the research. All authors read and approved the final manuscript.

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### Availability of data and materials

The data sets in this study are available from the corresponding author on reasonable request.

### Ethics approval and consent to participate

This study follows guidelines for the care and use of experimental animals established by the Animal Care and Use Committee of the University of Uyo for the purpose of control and supervision of experiments on animals. Informed consent is not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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