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BIOACTIVITY OF OIL PALM, *ELAEIS GUINEENSIS* (JACQ) (ARAECEAE) KERNEL OILS AGAINST *SITOPHILUS ORYZAE* (L.) (COLEOPTERA: CURCULIONIDAE): EXTRACTION METHODS AND TEMPERATURE EFFECT.

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ABSTRACT

Local and solvent-extracted palm kernel oils (LPKO and SPKO) were screened for bioactivity against *Sitophilus oryzae* (L.) in unpolished rice (*Oryza sativa* (L.)). Both oils were tested for toxicity to adults, and progeny inhibition at 0, 0.5, 1.0, 1.5, and 2.0 ml/50g of rice. Toxicity of mixtures of the oils: 1:1, 2:1(LPKO: SPKO), and 2:1 (SPKO: LPKO) was evaluated for synergism between LPKO and SPKO. Effect of temperature variation on bioactivity of individual oils was observed at 26, 28, 30, and 32^{o}C. Both oils killed adults, and inhibited F_1 progeny of *S. oryzae*. LPKO was more effective in killing adults and inhibiting F_1 progeny than SPKO. The 1.5ml/50g dose of LPKO caused 100% mortality at 48 h post-treatment. A 100% mortality was obtained with SPKO but at 2.0ml/50g in 72 h post-treatment. The order of toxicity of mixtures of PKOs was 2:1 LPKO: SPKO > 2:1 LPKO: SPKO > 2:1 SPKO: LPKO. Percent adult mortalities were higher in oil-mixtures than in individual oils which indicates synergistic effect of LPKO on SPKO. Toxicities of both oils increased with increasing temperatures. The results showed that LPKO possess some components of higher insecticidal potential, which if explored, could be useful in Integrated Pest Management of *S. oryzae*.

Keywords: Adult emergence, extraction methods, palm kernel oil, progeny inhibition, temperature, toxicity.

INTRODUCTION

Cereal grain is the major food source for man and livestock in developing countries (Rees, 2004). It is the main raw materials for food and pharmaceutical companies which provide employments for a greater percentage of the world's population (FAO, 2008; Tilman, *et al.*, 2002). Insect pests have been a serious constraint in grain production especially in developing countries and *Sitophilus* species are prominent among the major pests of cereals and cereal products (Longstaff, 1981a; Rees, 2004).

The biology of *Sitophilus oryzae* L. had been well documented in previous studies (Thomas *et al.*, 2002; Rees, 2004). The flight behaviour, voltinism and ability of all life stages to survive over a long period in wide range of temperatures and humidity make it a severe pest of cereal and cereal products world-wide (Campbell, 2005; Thomas *et al.*, 2002; Rees, 2004). Aside grain consumption which leaves infested grain brittle with emergent holes (Longstaff, 1981a), the respiratory activities and waste products of this insect increase temperature and moisture content of inter-granular air of stored grains which often result in grain damping and caking due to moisture migration (Mani *et al.*, 2001). Increase in temperature and moisture content of stored

grain often enhance development of spores of microorganisms with a resultant deposition of mycotoxins in grains. Economic loss due to grain infestation by *S. oryzae* is enormous, hence the need for the control of the beetle.

Synthetic chemical insecticides have remained the major tool for the control of insect pests in developing countries especially in Africa. Nigeria ranked first among West African countries in terms of pesticides importation from UK (Youm *et al.,* 1990). Majority of farmers in developing countries are ignorant, having little or no knowledge about handling and application of pesticides. Mishandling and improper applications of pesticides have caused deaths of humans and livestock, and many health problems (Ngowi *et al.,* 2007; Damalas and Eleftherohorinos, 2011). Insect resistance, toxicity to consumers and environmental hazards are serious problems facing the world due to misuse of pesticide (Koul *et al.,* 2008). Physical measures e.g the use of temperature and pneumatic conveyer (Johnson and Valero, 2003; Vadivambal *et al.,* 2010) are not within the reach of most farmers. These bottlenecks necessitate the search for a relatively safe and affordable alternative for control of stored grain pests.

Essential oils of many aromatic plants have been found toxic to *S. oryzae* (Thomas *et al.,* 2002; Paranagama *et al.,* 2004; Benzi *et al.,* 2009; Franz *et al.,* 2011; Chaubey, 2011; Hamed *et al.,* 2012) and other stored-products insect pests. Food oils have also been reported to be effective against stored-products insect pests (Ivbijaro *et al.,* 1985; Cory and Gerhard, 2010; Khani *et al.,* 2011; Deb and Borad, 2013). Khani *et al*. (2011) found oil components of *Piper nigrum* and *Jatropha curcas* toxic to *S. oryzae*. Hamed *et al*. (2012) found *Apium graveolens*, *Cinnamomum camphora*, and *Allium sativum* oils toxic to *S. oryzae*. Oil of leaves of *Cymbopogon citratus*, *Mentha* sp, and root of *Zingiber officinale* was found toxic to *S. oryzae* (Andrea *et al.,* 2011). Ivbijaro *et al*. (1985) also reported toxicity of oils of coconut, groundnut, and African palm to *S. oryzae*. These and many other researchers have presented essential oils as potential alternative biopesticides to synthetic chemical insecticides but most bioassays were done using individual plant oils that were solvent- extracted.

This present study investigates bioactivities of two oils extracted from kernels of *Elaeis guineensis* (Jacq) using traditional, and laboratory methods of extraction, against *S. oryzae*. Native to West and Central Africa (Hartley, 1988), oil palm, *E. guineensis* is a major source of red palm oil and palm kernel oil (Boateng *et al.,* 2008). The local palm-kernel oil of Nigeria differs from palm kernel oil extracted with organic solvent which has normal physical properties of vegetable oils. Local palm kernel oil is bitter to taste, dark-brown in color and has a very strong odor. It is used traditionally in cooking medicinal soup, and to expel intestinal worms. It was the major body and hair cream in the olden days, and still in use today as natural antibiotics to cure skin rashes and hair dandruffs in local areas. This study evaluates bioactivity of local palm kernel oil (LPKO) and solvent extracted palm kernel oil (SPKO), as well as their mixtures against *S. oryzae.*

MATERIALS AND METHODS

This research was conducted under laboratory conditions, 28 ± 2^{o}C and 75 ± 5% RH at the Federal University of Technology, Akure, Ondo State, Nigeria.

Plant materials

Uninfested brown rice was purchased from grocery store in Akure and kept in freezer at – 20^{o}C for 5 days to get rid of incipient infestation. Palm kernels were purchased from a local market in Ondo State, Nigeria and sun-dried for three days.

Insect culture

Rice weevil, *S. oryzae* was reared in the laboratory on disinfested brown rice in 1-L wide-mouthed Ball® Glass Mason jars. The choice of brown rice was to ensure the insects have access to natural nutrients to prevent growth barrier which might occur in polished artificially-coated rice. A fresh culture was started from an old culture of more than 10 generations by placing ~20 pairs (male and female) of 5-day-old *S. oryzae* in a glass jar

containing ~100 g of brown rice held at 30 ± 2 ^{o}C, 75 ± 5% RH, and a photoperiod of 12:12 h {L:D} (Adedire and Ajayi, 2003). The beetles were sexed using the key described by Rees (2004) and Halstead (1963). Adults that emerged from the fresh culture were used for bioassays.

Extraction of oil

Extraction of palm kernel oil with solvent

The palm-kernel was ground to palm-kernel cake using Hammer Mill Crusher (~16,000 – 23,000 feet per minute). *N*-hexane (analytical grade) was used to extract the oil in Soxhlet apparatus as described by Harbone (1984), and modified by Adedire and Ajayi (2003). Solvent was removed from the extract in rotary evaporator. The oil was exposed to air to remove traces of solvent (Ajayi, 2013). The solvent-extracted palm kernel oil (SPKO) was kept in dark bottle till used

Local palm kernel oil extraction

Unbroken palm kernel was put in local earthen pot and set on stone in a tripod form. Firewood was ignited under the pot of kernels to supply heat. Wooden cooking stick was used to turn the kernels continuously till the dry heat forced oil out of the kernels. The pot was removed and the content was poured into a palm-frond basket on another earthen pot to separate oil from kernels. Local palm kernel oil (LPKO) obtained was allowed to cool and kept in dark bottle till used.

PKO toxicity test on adult *Sitophilus oryzae* and F_1 progeny

Toxicity experiment was done with 100% individual oil, LPKO and SPKO, and mixtures of the oils in ratios 1:1 (LPKO: SPKO), 2:1 (LPKO: SPKO), and 2:1 (SPKO: LPKO). Experiments
were accomplished in covered plastic containers (10mm Ø, 5mm Height). Fifty grams of disinfested brown rice were weighed into each plastic plate. Four treatment dose-levels, 0.5, 1.0, 1.5, and 2.0 ml of each individual oil were applied on the rice, each dose was replicated five times. Mixtures of the oil were also tested against *S. oryzae* at the same dose-levels. The plates were agitated to ensure uniform coating of rice with the oils. Untreated rice served as controls. Ten pairs of 5-day-old *S. oryzae* (Ebadollahi and Mahboubi, 2011) were introduced into each plate. Number of dead insects were recorded at 24, 48, 72 and 96 h from the time of treatment.

All adult insects, dead or alive were removed at 96 h after treatment. The experiments were left in the laboratory undisturbed at above-mentioned laboratory conditions. Number of adults that emerged in each replicate were counted from 25 to 45 days after treatments.

Susceptibility of adult *Sitophilus oryzae* to PKOs at varying temperatures

Effect of temperature on susceptibility of adult *S. oryzae* to PKOs was studied. Fifty grams of rice were treated with 1ml of individual LPKO and SPKO. The treated rice were artificially infested with 10 pairs of *S. oryzae*. Four sets of each treatments were prepared and observed at four different temperatures, 26, 28, 30, and 32^{o}C for 24 h. Each treatment was replicated five times. Earlier studies had revealed optimum temperature for development of *S. oryzae* as 25 - 33^{o}C (Bank and Fields, 1995; Rees, 2004) hence the choice of the test temperatures. Number of dead insects were recorded at 24 h from the time of treatment.

Data Analysis

Experiments were completely randomized. Data obtained in percentages were arcsine transformed. ANOVA and Tukey's HSD Test at $\alpha = 0.05$ were performed on the transformed data analysed in SPSS version 20. Non-linear regression (Logarithmic model) was used to obtain LD_{50} of the oils. The LD_{50} values obtained were confirmed using Probit (Finney, 1952).

RESULTS

Toxicity of PKOs to *Sitophilus oryzae*

The local palm kernel oil (LPKO), solvent-extracted palm kernel oil (SPKO) and their mixtures varied in toxicity to *S. oryzae* at all doses tested (Tables 1 and 2). In the test with 100% individual oils, LPKO was more toxic to *S. oryzae* than SPKO. Toxicity increased with increase in exposure period (Table 1). A 100% insect death was obtained with 1.5ml of LPKO at 48 h after treatment (Table 1). SPKO also caused 100% insect death but at 2.0ml and 72 h

exposure time (Table 1). The LD_{50} of LPKO and SPKO based on mortalities at 24 h were 0.801 and 0.981ml/50g respectively (Fig. 1). Significant differences ($p < 0.05$) existed between mortalities of *S. oryzae* obtained with 1ml of LPKO and SPKO at 24 – 96 h post-treatment (Table 1). However, mortalities in LPKO and SPKO treatments were not significantly different with 0.5ml, at 24, 48 and 96 h ($p = 0.907$, 0.929 and 0.220), 1.5ml at 72, 96 h ($p= 0.717$, 1.000), and with 2.0ml 24 - 96 h (p ranged from 0.717-1.000) (Table 1). There were significant differences between mortalities of insects in untreated and mortalities in all treated seeds ($p < 0.05$) (Table 1).

All mixed oils caused a greater number of insect death at all dose levels. No insect death (0%) in untreated seeds (Table 2). higher number of the tested insect died at all doses of 2LPKO:1SPKO at all exposure periods (Table 2). Ratio 2:1 of SPKO: LPKO was least effective among oil mixtures tested (Table 2). LPKO: SPKO at ratio 2:1 caused a range of 75 – 100% insect death at 1.0ml dose and 24– 72 h. Similarly, 84 - 100% insect death was obtained with 1.5 ml of 1:1 of the oils. A 100% mortality was also obtained with ratio 2:1 of the oils at 1.5ml but not until 96 h. ANOVA showed significant differences ($p < .05$) between insect death in the three oil ratio treatments with time (24 h: $df_{12, 52} = 123.25$, $p = 0.0001$; 48 h: $df_{12, 52} = 158.86$, $p = 0.0001$; 72 h: $df_{12, 52} = 174.41$, $p = 0.0001$; 96 h: $df_{12, 52} = 299.29$, $p = 0.0001$). However, Tukey's HSD test revealed insignificant differences between insect death in different ratios and concentrations with time (Table 2). In general, percentages of insect deaths were greater in mixed oils than individual oil treatments (Tables 1 and 2). The LD_{50} of SPKO, 0.981ml/50g was about 0.2ml greater than the LD_{50} of 0.801ml/50g obtained for LPKO in this study (Fig. 1).

PKOs and *Sitophilus oryzae* F_1 progeny

The number of adults that emerged from PKOs treated seeds reduced with increasing concentrations and exposure periods (Fig. 2 and 3). In the test with individual LPKO and SPKO, adults emerged only at 0.5 and 1.0ml dose levels. Small number of adults emerged in LPKO-treated rice (37.8, and 12.8) compared to (57.8, and 26.2) in SPKO-treated rice at 0.5 and 1.0ml respectively (Fig. 2). The number of adults that emerged in untreated seeds ranged from 123.8 to 131.2 (Fig. 2). There were significant differences between the numbers of adults that emerged in oil-treated seeds, and untreated ($p < .05$) (Figs. 2 and 3). More adults emerged from mixed-oil-treated rice compared to individual oils at 0.5 and 1.0ml dose levels (Figures 2 and 3). The ratio 2LPKO:1SPKO had the least number of emerged adults (Fig. 3). Adults, though very small in number, 3.8 and 3.6, emerged at 1.5 and 2.0 doses respectively in 2SPKO:1LPKO-treated rice (Fig. 3).

Temperature and susceptibility of adult *Sitophilus oryzae* to PKOs

Adult mortality increased with increasing temperature (Fig. 4). Differences in percentages of insect mortality at 28, 30, and 32°C in LPKO treatments were not statistically significant ($p = 0.062$) but differed significantly ($p < 0.05$) from the mortality obtained at 26°C (Fig. 4). Mortalities in SPKO-treated rice varied significantly ($p < 0.05$) with temperatures except between 28 and 30 °C ($p = 0.510$). Tukey's HSD revealed significant differences between mortalities in LPKO and SPKO treatments at all temperatures ($p < 0.05$) as LPKO.

Table 1. **Cumulative mortality of adult *Sitophilus oryzae* exposed to local and solvent-extracted *Elaeis guineensis* kernel oils (Mean % ± SE.)**

		Exposure period (h)			
Dose (v/w)	**Oil Type**	**24**	**48**	**72**	**96**
0.5	LPKO	21.0^{e}±1.0	29.0^{e}±2.9	48.8^{c}±3.0	56.8^{c}±5.7

	SPKO	16.8^{e}±1.3	26.0^{e}±1.9	34.3^{d}±2.3	44.8^{c}±3.6
1.0	LPKO	61.0^{c}±4.5	69.0^{c}±1.9	71.3^{b}±3.5	75.4^{b}±3.2
	SPKO	49.0^{d}±1.8	53.0^{d}±2.0	53.0^{c}±3.6	57.4^{c}±3.6
1.5	LPKO	96.0^{a}±1.9	100.0^{a}±0.0	100.0^{a}±0.0	100.0^{a}±0.0
	SPKO	76.0^{b}±2.1	79.0^{b}±1.9	93.6^{a}±3.2	97.8^{a}±1.4
2.0	LPKO	100.0^{a}±0.0	100.0^{a}±0.0	100.0^{a}±0.0	100.0^{a}±0.0
	SPKO	82.0^{b}±2.5	98.0^{a}±1.2	100.0^{a}±0.0	100.0^{a}±0.0
0.0	Control	0.0^{f}±0.0	0.0^{f}±0.0	8.0^{e}±3.4	10.0^{d}±2.2

Means followed by the same letter (s) within the same column are not significantly different at $\alpha = 0.05$ using ANOVA and Tukey's HSD Test.

Table 2. **Cumulative mortality of *Sitophilus oryzae* exposed to mixtures of local and solvent-extracted *Elaeis guineensis* kernel oils (Mean % ± SE.)**

		Exposure Period (h)			
Dose (v/w)	**Oil Type**	**24**	**48**	**72**	**96**
	1LPKO:1SPKO	30.0^{f} ± 1.6	49.0^{d} ± 2.9	71.0^{c} ± 2.9	83.0^{c} ± 2.0
0.5	2LPKO:1SPKO	47.0^{e} ± 2.5	61.0^{c} ± 4.3	72.0^{c} ± 2.9	82.0^{c} ± 2.5
	2SPKO:1LPKO	25.0^{f} ± 2.2	41.0^{d} ± 1.9	60.0^{d} ± 3.5	70.0^{d} ± 4.2
	1LPKO:1SPKO	56.0^{e} ± 2.9	70.0^{c} ± 2.2	78.0^{bc} ± 2.5	94.0^{ab} ± 1.9
1.0	2LPKO:1SPKO	75.0^{cd} ± 4.2	90.0^{ab} ± 1.6	100.0^{a} ± 0.0	100.0^{a} ± 0.0
	2SPKO:1LPKO	48.0^{e} ± 2.5	63.0^{c} ± 2.5	76.0^{c} ± 3.4	88.0^{bc} ± 1.2
	1LPKO:1SPKO	84.0^{abcd} ± 1.9	94.0^{ab} ± 1.9	98.0^{a} ± 1.2	100.0^{a} ± 0.0
1.5	2LPKO:1SPKO	87.0^{abc} ± 4.1	96.0^{a} ± 1.9	100.0^{a} ± 0.0	100.0^{a} ± 0.0
	2SPKO:1LPKO	71.0^{d} ± 3.7	83.0^{b} ± 3.4	87.0^{b} ± 3.0	100.0^{a} ± 0.0
	1LPKO:1SPKO	94.0^{ab} ± 1.9	99.0^{a} ± 1.0	100.0^{a} ± 0.0	100.0^{a} ± 0.0
2.0	2LPKO:1SPKO	97.0^{a} ± 2.0	100.0^{a} ± 0.0	100.0^{a} ±0.0	100.0^{a} ± 0.0

	2SPKO:1LPKO	82.0 ± 2.5	93.0^{ab} ± 2.5	100.0^{a} ± 0.0	100.0^{a} ± 0.0
0.0	Control	0.0^{g}±0.0	0.0^{e}±0.0	0.0^{e}±0.0	0.0^{e} ± 0.0

Means followed by the same letter (s) within the same column are not significantly different at $\alpha = 0.05$ using ANOVA and Tukey's HSD Test.

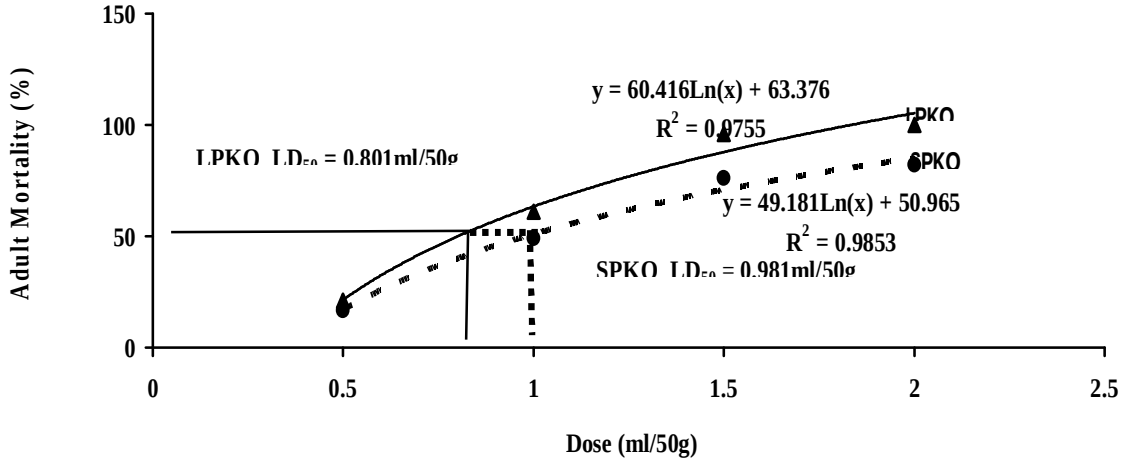

Figure 1: LD_{50} of LPKO and SPKO tested against *Sitophilus oryzae* at 24 h exposure period.

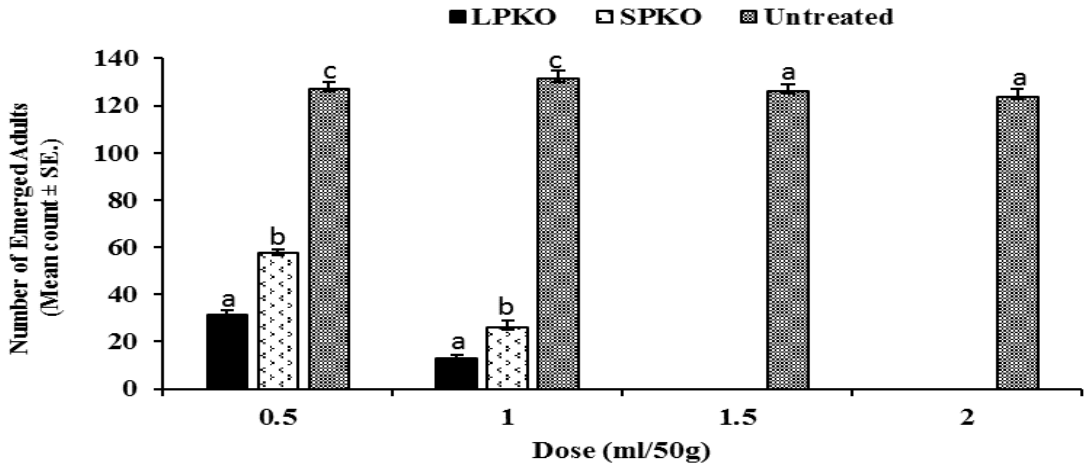

Figure 2: Number of adult *Sitophilus oryzae* that emerged from rice treated with individual local, and solvent extracted PKO.

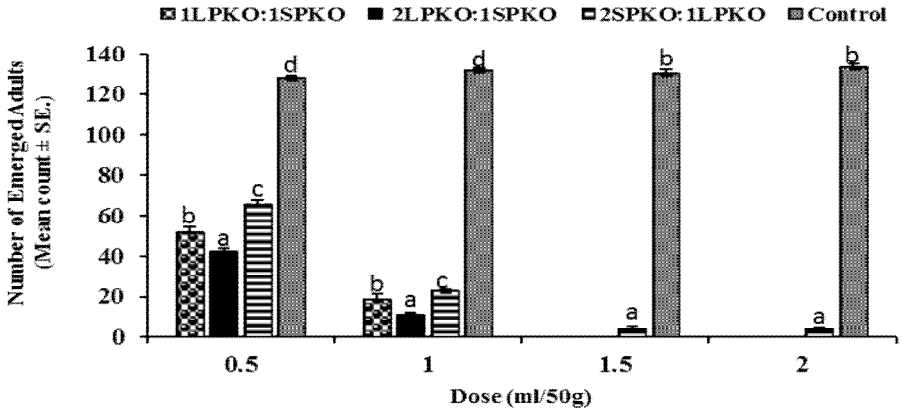

Figure 3: Number of adult *Sitophilus oryzae* that emerged from rice treated with mixture of local and solvent extracted PKO

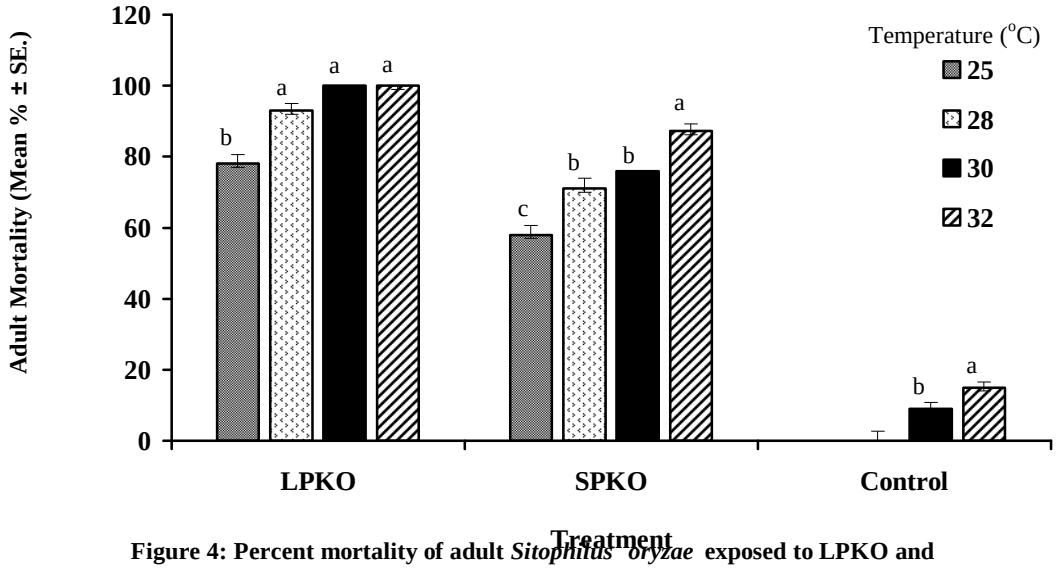

Figure 4: Percent mortality of adult *Sitophilus oryzae* exposed to LPKO and SPKO at different temperatures.

DISCUSSION

Local (LPKO) and solvent-extracted (SPKO) palm kernel oils tested for bioactivity against *Sitophilus oryzae* showed varying degrees of toxicity against adult insects and inhibited F_1 progeny. LPKO was more toxic to adult insects, and also had higher inhibitory effect on F_1 progeny than SPKO. This signifies the possibility of LPKO having some components that may not be in SPKO which probably might have been formed during extraction process. Insect mortalities in mixed oil treatments were far greater than the mortalities obtained in individual oils at all dose-levels. The mixture that had a greater proportion of LPKO, 2LPKO:1SPKO was most toxic to *S. oryzae* among the mixed oil which possibly indicates synergistic effect between LPKO and SPKO.

Insect mortality increased with increasing temperature. Basically, increase in temperature causes expansion in molecular structures of the body and increase in the rate at which biochemical reactions occur particularly in insects (Bowler, 1987; Lisa, 2000; Michael *et al*., 2004). The higher temperatures used in this study may have caused expansion in spiracles and cuticle of the beetle allowing for more of the oil to penetrate into its body, and again increase

rate of biochemical reactions between the oils and the body of the beetles hence, a greater mortality. The insignificant differences between mortalities of the beetles obtained with LPKO at 28 – 32°C signals the possibility that LPKO components are more stable than SPKO at the stated temperature range. The stability in the environment might be due to dry-heat extraction process of LPKO. Local extraction method involves heating unbroken kernel at a very high temperature to force the oil out of the kernels. Unlike extraction of SPKO with *n*-hexane that was done at 65°C in conical flask which precludes oxygen, the dry heating of kernels to a very high temperature in oxygenated condition (open air) might, in addition to oxidation, have caused some other chemical reactions to take place with a resultant formation of compounds that were not naturally present in the kernels. Saturated fatty acids at 2-position of triacylglycerols, conjugated fatty acids and polycylic aromatic compounds are some of the toxic products of inter- or intra-esterification during extreme heating (Macaire *et al.,* 2010). This may account for the differences in colour, taste, odour, and ultimately, the toxicity of LPKO to *S. oryzae* found in this study. Generally, PKO contains high percentage (88%) of saturated fatty acids: lauric, myristic, palmitic, and about 15% oleic acid as major fatty acid components (Akpanabiatu *et al.,* 2001; Mukherjee and Mitra, 2009). Toxicity of lauric, myristic, and oleic acids to adult insect pests and their developmental stages had been reported (Don-Pedro, 1990; Sims *et al.,* 2014). Potassium salts of fatty acids lauric, myristic, and oleic acids are used as insecticides, acaricides, herbicides and algaecides on crops, and plants of interest. Also, ammonium salts of fatty acids are also used as a rabbit and deer repellent on crops (Don-Pedro, 1990, 1996; US EPA, 1992). Therefore, the bioactivity of PKOs reported in this study might be due to the combined effects of the fatty acid components and their derivatives.

Previous studies have reported the toxicity of essential oils of many plants including oil of palm fruit to *S. oryzae* and other stored products pests in general (Debjan and Prakash, 1993; Paranagama *et al.,* 2004; Benzi, *et al.,* 2009; Chaubey, 2011 and Ajayi, 2013) but little is known about toxicity of palm kernel oil to stored products insect pests (Law-Ogbomo and Enobakhare, 2006). The results of this study agrees with the 55-93% mortality of *Sitophilus zeamais* (L) in PKO-treated maize reported by Law-Ogbomo and Enobakhare (2006).

CONCLUSION

Local and solvent-extracted PKOs are toxic to adults *S. oryzae*. The oils had high reduction effect on emergence of F_1 progeny of the insect. LPKO was more effective at killing adult *S. oryzae* and inhibiting F_1 progeny than SPKO. Bioactivity of LPKO was stable at 28 – 32°C. Toxicity of mixed oil revealed synergistic effect of LPKO on SPKO. The overall results indicated that LPKO could have greater insecticidal bioactivity than SPKO. Further research is necessary to harness the possible inherent insecticidal potential of LPKO and SPKO for control of stored product pests.

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