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Chemical composition and toxicity of *Jatropha curcas* seed oil against *Sitophilus zeamais* Motschulsky as affected by pre-extraction treatment of seeds

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ABSTRACT

This study evaluated the chemical composition and toxicity of fixed oils obtained from *Jatropha curcas* seeds pre-treated with different methods (cooking, roasting and raw) against maize weevil, *Sitophilus zeamais* Motschulsky. Palmitic acid was the predominant compound, being 86.60, 69.98 and 54.04%, in oil obtained from cooked, raw and roasted seeds, respectively. Glycerol 1-palmitate was identified in all oil samples (13.40. 16.43 and 27.06% for the oil extracted from cooked, raw and roasted seeds, respectively). Other compounds identified in oils obtained from raw seeds and roasted seeds were palmitoleic acid (2.36 and 2.16%, respectively), linoleic acid (3.13 and 5.80%, respectively) and 9 Octadecenal, (Z)- (8.10 and 10.94%, respectively). At the exposure of the weevils to 200 µl/L air for 24 h, weevil mortality observed in oil obtained from roasted seeds (84.68%) was significantly ($p < 0.05$) higher than 40.28 and 47.80% observed in oils obtained from cooked and raw seeds, respectively. In contact toxicity bioassay, application of 1.50 µl/cm^2 for 3 h evoked 47.52, 46.96 and 47.36% mortality for oil obtained from roasted, cooked and raw seeds, respectively; being higher than the values observed in untreated control and n-hexane. According to these results, pre-extraction seed treatment affected the chemical composition and toxicity of the oil against *Sitophilus zeamais*.

1. Introduction

Maize, *Zea mays* L., belongs to the family Poaceae. It is a popular staple food grain across the globe, and a major feedstuff ingredient for livestock in many developing countries. The maize grain has many food (grain, flour, syrup, and oil) and non-food usages (cosmetics, adhesives, starch, oil, paints and varnishes) (Badu-Apraku and Fakorede, 2017; De Groote et al., 2017; Ekpa et al., 2018). A major constraint to its all-season availability is postharvest pest problems, with insects, birds, rodents and pathogenic microorganisms being the main pests.

The maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Dryophthoridae), is one of the most serious cosmopolitan insect pests of stored cereal grain in tropical and sub-tropical regions (Obeng-Ofori and Amiteye, 2005; Babarinde et al., 2008). It can damage both maize on the cobs and shelled grains, by reducing them to powder and creating holes on the grains. The maize weevil can also cause loss in germination ability and nutritional quality. Apart from cereals, it can also infest non-cereals and other processed cereals products like pasta, processed and dried yam and cassava (Nwana, 1993; Babarinde et al., 2013a,b; Trematteria and Savoldelli, 2014).

Jatropha curcas (family Euphorbiaceae) is cultivated in central and south America, Southeast Asia, India and Africa (Gübitz et al., 1999). It can be propagated by stem cutting or seed. It is not browsed by ruminants and often planted closely on the boundary to form a hedge and protect the field's erosion. It can grow well under adverse weather because of its tolerance to high temperatures, low fertility requirements and moisture demands (Kaushik et al., 2007). Two accessions of *J. curcas* are known, which are toxic and non-toxic accessions (Aregheore et al., 1998; Francis et al., 2013). *Jatropha* oil from toxic accession may not be used for nutritional purposes without detoxification; hence, its use as energy or fuel source. Seeds are often processed into cake as a component of animal feed. In order to obtain the best *Jatropha* cake that can be

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incorporated into livestock feed, several seed treatments have been evaluated (Kumar et al., 2010; Ojediran and Emiola, 2012; Makker, 2016). Usually, fixed oil is obtained as a major by-product in the cake production; and the oil can be explored for the protection of stored products against insects and microbial pests.

Although fixed oils may not be as effective as some marketed synthetic pesticides, their better ecological safety than the synthetic pesticides makes them candidates of research interest to eco-toxicologists and pest control specialists. Several studies have been conducted on the pesticidal potentials of several *J. curcas* formulations. For instance, the insecticidal properties of the oil against cowpea seed bruchid, *Callosobruchus maculatus,* and desert locust, *Schistocerca gregaria,* have been documented (Adebowale and Adedire, 2006; Bashir and El Shafie, 2013), while the seed dust was also reported to be toxic against maize weevil, *S. zeamais* (Ukpai et al., 2017). The toxicities of the leaf bark and root extracts against housefly, *Musca domestica* (Chauhan et al., 2015) and the extracts of leaf, bark, seed, seed coat and roots against diamond back moth and *Helicoverpa armigera* (Krishnananda et al., 2017), and the leaf extract against *Culex quinquefasciatus* (Kovendan et al., 2011) have also been reported. In addition to the reported toxicity of *J. curcas* against various pests, various researchers have reported the toxicity of powder formulation (Silva et al., 2012; Suleiman et al., 2012; Ojiako et al., 2014) and extracts (Ohazurike et al., 2004; Asmanizar and Idris, 2012; Silva et al., 2012; Jide-Ojo et al., 2013) obtained from different parts of the plant against the genus *Sitophilus.*

It has been reported that the bioactivity of *Hoslundia opposita* essential oil against *S. zeamais* was influenced by the pre-extraction treatment of its leaves (Babarinde et al., 2017a,b).

Despite the vast volume of published works on the insecticidal properties of *J. curcas* seed oil, attention has not been drawn on the impact of the pre-extraction treatment of seeds or the extraction method on the bioactivity of the oil. Therefore, this study was designed with the following objectives: (a) to evaluate the effect of different pre-extraction treatments of *Jatropha curcas* seed on the efficacy of the seed oil as toxicant against maize weevil, *Sitophilus zeamais,* and (b) to evaluate the chemical composition of the oil extracted from different pre-extraction seed treatment via chromatographic method.

2. Materials and methods

2.1. Mass rearing of insects

Sitophilus zeamais adults were obtained from an infested maize sample obtained from Sabo Market, Ogbomoso. They were introduced into kilner jars containing clean un-infested seeds of Pajo white, a local maize cultivar. The jars were maintained in the laboratory under ambient temperature ($28 \pm 2°C$) and relative humidity ($65 \pm 5\%$). The dead insects were removed from the culture after oviposition and the setup kept until the emergence of F_1 generation. The F_1 adults were used for subsequent re-infestation to maintain the insect culture throughout the period of the experiment (Babarinde et al., 2008).

2.2. Pre-extraction seed treatment and Jatropha seed oil extraction

Seeds of local variety of toxic *Jatropha curcas* were obtained from Kano, Kano State, Nigeria. Dirt and extraneous matters were separated from the seeds. Thereafter, the kernels were manually separated from the testa. The seed lot (3 kg) was then divided into three equal portions and each portion was subjected to three different treatments as earlier described (Ojediran and Emiola, 2012). The methodology is briefly described as follows: (a) Roasting: A portion (1 kg) of the seeds was roasted in frying pan at 120°C for about 20 min till the seeds became crispy and turned brown (b) Cooking: Another 1 kg of the seed was boiled in 3 L distilled water at 120°C for 30 min (c) Raw: The third portion (1 kg) was left raw without treatment. The seed lots treated with each method were subjected to mechanical extraction method using hydraulic press (Babarinde et al., 2011; Subroto et al., 2015).

2.3. Chromatographic procedure

Gas Chromatography Mass Spectrometry (GCMS) conditions used for the analysis of the oils followed previous description (Babarinde et al., 2016). An AGILENT (19091S–433HP–5MS) GC interfaced with a VG Analytical 70–250 S, a double focusing mass spectrometer was used. Helium was used as the carrier gas at 1.5 ml/min. The MS operating conditions were as follows: ionization with an ion trap detector in full scan mode under electron impact ionization (EI) at 70 eV, ion source temperature 230 °C; interface temperature 250 °C, scan range, 40–700 m/z. The GC was fitted with a 25 m × 0.25 mm, fused silica capillary column coated with CP-Sil 5. The film thickness was 0.15 µm. The MS operating conditions were identical with those of GC analysis. The MS data were acquired and processed by online desktop computer equipped with disk memory. The percentage compositions of the oil were computed in each case from GC peak areas. The identification of the components was based on the retention indices (determined relative to the retention times of series of n-alkanes) and mass spectra which were compared with those of authentic samples and with data from literature (Adams, 2001; NIST, 2010) as reported by previous authors (Babarinde et al., 2017b; De Carlo et al., 2018).

2.4. Fumigant toxicity bioassay

Fumigant toxicity bioassay was carried out according to the method previously described (Babarinde et al., 2017b) with some modifications. Briefly, No. 1 Whatman filter paper (approximately 8 cm^2) was folded and glued to the inner surface of the fumigation chamber's lid. Varying doses (50, 100, 150 and 200 µ1/L air) of each sample of the *J. curcas* oil was applied on the filter paper and the chamber was closed. Ten minutes later, ten mixed-sex *S. zeamais* adults (1- to 3-day old) were introduced into each fumigation chamber, which was covered with the lid. The experiment was replicated five times. Mortality data were taken at 12 and 24 h after treatment (HAT).

2.5. Contact toxicity bioassay

Contact toxicity was carried out by applying varying doses (0.30, 0.60, 0.90, 1.20 and 1.50 µ1/cm^2) of the oil extracted from each pre-extraction *J. curcas* seed treatment on 9 cm diameter No. 1 Whatman filter paper inside 9 cm diameter Petri-dishes. Each dose was separately dissolved in 10 µ1 n-hexane and applied to the filter paper. Application of each oil and n-hexane on filter paper was done with the aid of separate micro-applicator. Two controls (untreated filter paper and filter paper treated with 10 µ1 n-hexane) were included in the bioassay. Ten mixed-sex *S. zeamais* adults (1- to 3-day old) were separately introduced into the oil-treated, n-hexane-treated and untreated filter papers and the setup was covered with the Petri dish lids. The experiment was replicated five times and data on mortality were collected at 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 HAT.

2.6. Experimental design and statistical analyses

Each of the bioassays was laid out in Completely Randomized Design and data were subjected to Analysis of Variance (Ojo and Ogunleye, 2013) and significant means were separated using Student-Newman-Keuls (SNK) test at 5% probability level. All data analyses were conducted with the aid of Statistical Package for Social Sciences (SPSS) Software.

3. Results

3.1. Chemical composition of Jatropha curcas seed oil obtained from different pre-extraction seed treatments

There was quantitative and qualitative variations in the chemical compositions of the oils obtained from the seeds that were exposed to different pre-extraction treatments. Regardless of the pre-extraction treatment to which the seeds were subjected, saturated fatty acid was the predominant group being 86.60, 69.98 and 54.04% for the oil extracted from cooked, raw and roasted seeds, respectively. Palmitic acid was the predominant compound (86.60, 69.98 and 54.04%, in oil obtained from cooked seeds, raw seeds and roasted seeds, respectively). Seeds that were cooked before oil extraction had Glycerol 1-palmitate (13.40%) as the second compound, while it was also identified in the oil extracted from raw seeds (16.43%). Other compounds identified in the oils obtained from raw seeds and roasted seeds were palmitoleic acid (2.36 and 2.16%, respectively), Linoleic acid (3.13 and 5.80%, respectively) and 9 Octadecenal, (Z)- (8.10 and 10.94%, respectively) (Table 1).

3.2. Fumigant toxicity of Jatropha curcas seed oil obtained from different pre-extraction seed treatments

Regardless of the pre-extraction seed treatment, toxicity of *J. caucus* seed oil against *S. zeamais* was exposure-period dependent. At 12 HAT, highest percentage mortality was observed in weevils treated with 200 µ1/L air of oil obtained from roasted seeds which was significantly ($p < 0.05$) higher than the values obtained in 200 µ1/L air of oils obtained from cooked seeds (15.64%) and raw seeds (38.48%) and the mortality observed in other lower oil doses, regardless of the pre-extraction seed treatments (Fig. 1a). At 24 HAT, oil obtained from roasted seeds gave 84.68% mortality which was significantly higher than 11.96–47.80% mortality observed in all other lower doses of oils regardless of the pre-extraction seed treatments (Fig. 1b).

3.3. Contact toxicity of Jatropha curcas seed oil obtained from different pre-extraction seed treatments

The result of the contact toxicity bioassay is presented in Table 2. As observed in the fumigant toxicity bioassay, contact toxicity also progressed with exposure period. At 0.5 HAT, application of the oil extracted from the roasted seeds at 1.50 µl/cm^2 evoked 18.88% mortality which was significantly higher than 0.00% observed in the controls, but not different from 3.68 and 7.36% mortality obtained from the oils extracted from the raw and cooked seeds, respectively. The same trend was observed at 1.0–2.5 HAT. At 3.0 HAT, 47.52, 46.96 and 47.36% mortality values observed in the weevils treated with the oil extracted from roasted, cooked and raw seeds, respectively were not significantly different (Table 2).

4. Discussion

The result of the chromatographic analysis of the oils indicates that regardless of the pre-extraction treatment of the seeds, *J. curcas* seed oil was saturated fatty acid type, being predominated by Palmitic acid. Palmitic and Linoleic acid have been identified as constituents of *J. curcas* seed oil from Nigeria. Apart from the fatty acids, monoacylglycerol and diacylglycerol were identified in the *J. curcas* seed oil (Adebowale and Adedire, 2006). Palmitic acid, Palmitoleic acid and Linoleic acid were identified in tropical *J. curcas* seed oil (Gübitz et al., 1999), while Palmitic acid and Linoleic acid were identified in the seed oil from Asia (Pramanik, 2003; Berchmans and Hirata, 2008; Akbar et al., 2009). However, from four Mexican provenances, the fatty acids identified in *J. curcas* seed oil were Oleic acid, Palmitic acid and Linoleic acid (Martınez-Herrera et al., 2006). Factors responsible for the variations in the chemical composition could be the differences in the studied *J. curcas* varieties, geographical influence, soil nutrients and the oil extraction methodology.

The oil extracted from cooked seeds had the least biodiversity in terms of the number of the identified compounds. This could be due to chemical reaction of the oil under high boiling temperature in the presence of water. Marr and Ingraham (1962) reported that the proportion of unsaturated fatty acids increases as the growth temperature of *Escherichia coli* was lowered. Continuous exposure of fat/oil to high temperatures in the presence of air causes it to undergo hydrolysis, oxidation and polymerisation, leading to deterioration in quality, thereby causing changes in not only the sensory but also the nutritional properties (Zhang et al., 2015). Heating has been reported to cause the reduction of the magnitude of unsaturated fatty acids in edible fats/oils, while the level of saturated fatty acids increased with temperature increase (Noble et al., 1967). In the samples of corn oil, cottonseed oil, and lard heated at 200°C, a preference for the hydrolysis of the shorter chain and the unsaturated acids was noticed (Zhang et al., 2012). Volatile Aldehydes were significantly formed from the unsaturated fatty acids when soybean oil was used as the frying medium of wheat dough and chicken breast meat frying (Bhardwaj et al., 2016).

All *J. curcas* seed oils were toxic against *S. zeamais.* However, the results of the fumigant toxicity indicate that the oil extracted from the roasted *J. curcas* seed was more toxic than the oils obtained from the seeds given other pre-extraction treatments. Toxicity progressed with exposure period in the fumigation chamber, since the weevils had no escape route throughout the experimental period. Hence, the weevils were compelled to cumulatively absorb the dose of the toxic oil into their body system via their spiracles. This result follows the observations made by previous researchers. For instance, the cowpea bruchid,

Table 1
Chemical composition of fixed oil obtained from *Jatropha curcas* seed pre-treated by different methods.

S/N	Compounds	Class	Formula	Pre-extraction seed treatment {% Composition, Retention Time (min)}					
				Cooked seeds		Raw seeds		Roasted seeds	
1	Palmitoleic acid	Monounsaturated fatty acid	$C_{16}H_{30}O_2$	-	-	2.36	27.38	2.16	27.31
2	Palmitic acid	Saturated fatty acid	$C_{16}H_{32}O_2$	86.60	28.16	69.98	28.25	54.04	28.11
3	Linoleic acid	Polyunsaturated fatty acid	$C_{18}H_{30}O_2$	-	-	3.13	38.61	5.80	38.58
4	9 Octadecenal, (Z) -	Fatty aldehyde	$C_{18}H_{34}O$	-	-	8.10	39.70	10.94	38.64
5	Glycerol 1-palmitate	Glyceride	$C_{19}H_{38}O_4$	13.40	39.40	16.43	39.47	27.06	39.45
	Group (%)								
	Monounsaturated fatty acid			0.00		2.36		2.16	
	Saturated fatty acid			86.60		69.98		54.04	
	Polyunsaturated fatty acid			0.00		3.13		5.80	
	Fatty aldehyde			0.00		8.10		10.94	
	Glyceride			13.40		16.43		27.06	
	Total			100.00		100.00		100.00	

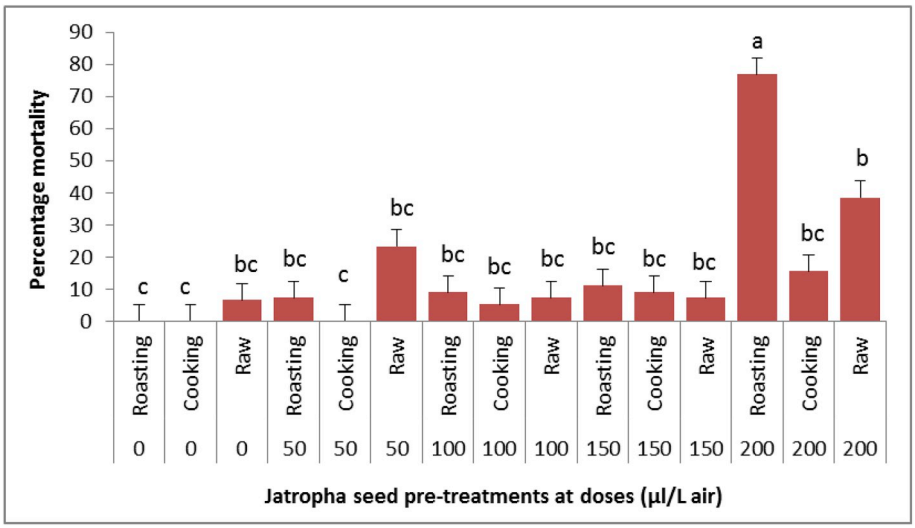

Fig. 1a. Fumigant toxicity of *Jatropha curcas* seed oil obtained from seeds given different pre-extraction treatments against *Sitophilus zeamais* at 12 h post oil application. {Means with different alphabets are significantly different using Student-Newman-Keuls (SNK) at 5% significance level.}

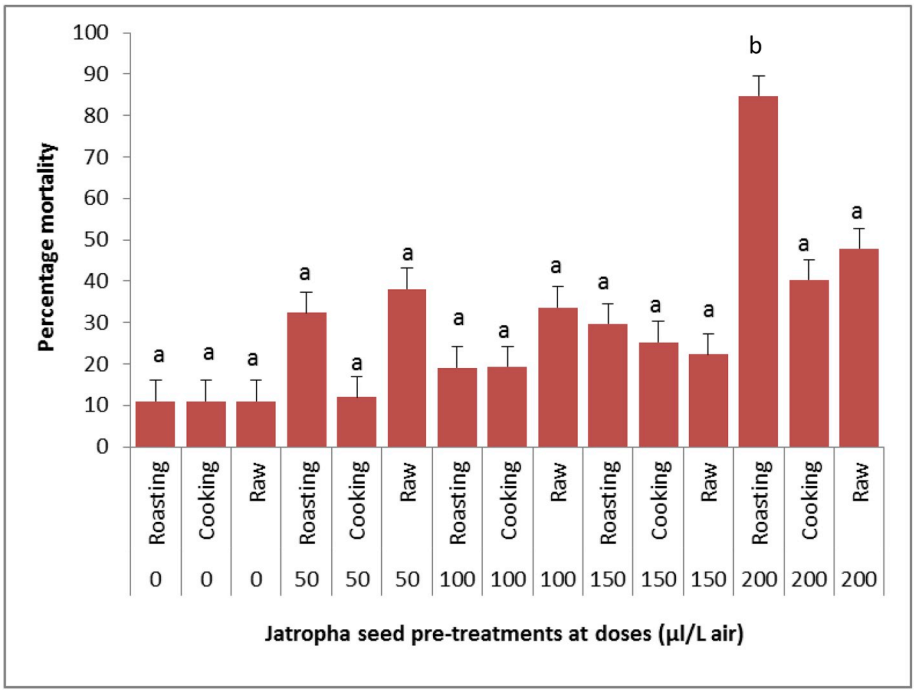

Fig. 1b. Fumigant toxicity of *Jatropha curcas* seed oil obtained from seeds given different pre-extraction treatments against *Sitophilus zeamais* at 24 h post oil application. {Means with different alphabets are significantly different using Student-Newman-Keuls (SNK) at 5% significance level.}.

C. maculatus, exposed to *J. curcas* seed oil suffered higher toxicity with increase in the exposure period (Adebowale and Adedire, 2006). In another experiment, higher percentage mortality was observed in red-rust flour beetle, *Tribolium castaneum* exposed to *Ricinus communis* seed oil at longer exposure period (Babarinde et al., 2011). Also, Bashir and El Shafie (2013) reported the insecticidal and antifeedant effect of *J. curcas* seed oil against the desert locust, *S. gregarious.* Apart from the oil, extracts and powder of *J. curcas* have also been reported to be toxic against *S. zeamais* (Ohazurike et al., 2004; Asmanizar and Idris, 2012; Silva et al., 2012; Jide-Ojo et al., 2013).

The result of the contact toxicity did not follow the same trend as the result of fumigant toxicity. Although all the oils were toxic, there was no disparity in the toxicity of the oils regardless of the pre-extraction treatment to which the seeds were subjected. Besides, the mortality values observed in contact toxicity were comparatively lower than the values observed in the fumigant toxicity bioassay. It was possible that parts of the bioactive compounds of the oils which were dissolved in n-hexane were absorbed by the filter paper; hence, could not be available for the envisaged bioactivity. This result implies that the bioactivity of oil against insect pests partly depends on the bioassay type and the applied methodology.

It was observed that the oil extracted from the cooked seeds evoked comparatively lower fumigant toxicity than the oil observed from roasted seeds. The GCMS result also showed that the latter had lower number of constituent compounds (two). Although, the effect of the individual constituent compounds was not investigated in this study, it was possible that the comparatively lower fumigant bioactivity against the weevil, observed in the oil that was obtained from cooked seed was

Table 2
Contact toxicity of *Jatropha* caucus seed oil obtained from seeds given different pre-extraction treatments against *Sitophilus zeamais*.

Jatropha Seed Treatment	Dosage (μl/cm^2)	Time after exposure (hours)					
		0.5	1.0	1.5	2.0	2.5	3.0
	Control	0.00b	0.00b	0.00b	0.00e	0.00e	0.00c
	n-Hexane	3.68ab	5.32a	5.32ab	9.00cde	9.00cde	18.00b
Roasting	0.30	0.00b	0.00b	3.68ab	3.68de	3.68de	25.96ab
	0.60	7.36b	9.00a	9.00ab	14.32bcde	14.32bcde	25.84ab
	0.90	3.68a	11.04a	19.64ab	19.64abcde	19.64abcde	39.12ab
	1.20	7.36b	7.36ab	15.64ab	17.28abcde	17.28abcde	37.64ab
	1.50	18.88a	23.48a	32.52a	36.64abc	36.64abc	47.52a
Cooking	0.30	7.36ab	11.04a	11.04ab	22.20abcde	22.20abcde	30.00ab
	0.60	7.36ab	12.68a	20.04ab	28.80abcd	28.80abcd	32.76ab
	0.90	3.68ab	10.32a	23.60ab	33.68abc	33.68abc	36.48ab
	1.20	5.32ab	19.16a	32.00a	42.68a	42.68a	46.96a
	1.50	7.36ab	25.8a	28.64ab	41.48ab	41.48ab	46.96a
Raw	0.30	0.00b	0.00b	9.00ab	21.68abcde	21.68abcde	30.84ab
	0.60	0.00b	3.68b	12.68ab	16.36abcde	16.36abcde	33.96ab
	0.90	7.36ab	7.36ab	14.72ab	24.32abcde	24.32abcde	30.12ab
	1.20	0.00b	9.00ab	19.36ab	26.72abcde	26.72abcde	35.44ab
	1.50	3.68ab	17.20a	32.48a	35.44abc	35.44abc	47.36a

Means with different alphabets along the column are significantly different using Student-Newman-Keuls (SNK) at 5% level of significance.

due to the chemical composition of the oil. Babarinde et al. (2016) postulated that Palmitic acid and Linoleic acid were responsible for the toxicity of neem seed oil against *Dermestes maculatus,* a storage pest of dried fish. Similarly, Palmitic acid, Linoleic acid and Palmitoleic acid, among others, have been identified in several fixed oils which exhibited toxicity against various insect pests (Rahuman et al., 2008; Suqi et al., 2014; Eyol et al., 2017). It has been reported that the essential oils with multiple chemical compounds exhibited higher bioactivity due to the synergistic effect of the constituent compounds (Babarinde et al., 2017a, b; 2018). Similar observations was made by Zekri et al. (2016) who evaluated the insecticidal effects of the hydrosols of *Mentha suaveolens and M. pulegium* against *Toxoptera aurantii,* an aphid, which attacks citrus. Acetylcholinesterase (AChE) has been identified as the main site of action of Palmitic acid in mosquito larvae. The mechanism of toxicity of Linoleic acid might be on both octopaminergic and AChE receptors (Zekri et al., 2016). Therefore the mode of action of the fixed oils against *S. zeamais* was possibly an influence on AChE and octopaminergic receptors.

5. Conclusion

In conclusion, the pre-extraction seed treatment had significant effect on the chemical composition of the obtained oils from the seeds of *J. curcas.* Seeds roasted and raw seeds yielded oils with five chemical compounds, while the oil extracted from cooked seed had two compounds. In the same vein, oil obtained from cooked seeds had comparatively lower bioactivity than oils obtained from other pre-extraction seed treatments. Roasting of seeds of *J. curcas* for about 20 min until crisps is therefore recommended as a pre-extraction treatment for the production of the oil meant for insecticidal purpose. Other pre-extraction seed treatments can be evaluated and be compared with the results obtained in this study.

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