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Bio-efficacy of *Azadirachta indica* A. Juss oil extracted from sun- and shade-dried seeds against two stored-product beetles

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Abstract

Azadirachta indica is a source of highly effective botanical insecticides, with Azadirachtin A as the major active principle. However, it has been contended that sun-drying reduces its Azadirachtin A content and insecticidal efficacy. This work investigated the Azadirachtin A content, fatty acid contents, adult toxicity, F_1 progeny inhibition and damage reduction of *A. indica* oils from sun-dried kernels, shade-dried kernels, sun-dried seeds and shade-dried seeds, against *Callosobruchus maculatus* Fab. (Coleoptera: Chrysomelidae) and *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae), serious storage pest of cowpea and maize, respectively. Analysis by liquid chromatography-electrospray ionization-tandem mass spectrometry showed that the sun-dried seed, but not the sun-dried kernel oil gave less Azadirachtin A amount 2.89 ± 0.17 g/kg compared to the other drying regimes (3.09 ± 0.09 – 3.69 ± 0.16 g/kg). Gas chromatography equipped with a flame-ionisation detector analysis revealed the presence of palmitic acid, stearic acid, oleic acid and linoleic acid, as the major fatty acids in the oils, with similarity in their concentrations among the four drying regimes. The adult toxicity assay showed that *C. maculatus* was more susceptible to the *A. indica* oils than *S. zeamais*, but without differences linked to the drying regime. All the oils completely suppressed progeny production and grain damage, irrespective of the drying regime and insect species. Our results disagree with the contention that sun-drying diminishes the Azadirachtin A content and insecticidal efficacy of *A. indica*. Therefore, sun-drying could be adopted by farmers because it may speed up processing of seeds and minimize attacks by aflatoxin producing fungi.

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Introduction

Over the past decades, synthetic chemical insecticides have played a significant role in modern agricultural pest management (Guo *et al.*, 2014). Their repeated use over the years has led to the evolving of resistance in pest populations and fostered environmental and human health concerns. These problems have highlighted the need for the development of new types of selective insect-control alternatives (Lee *et al.,* 2001). Currently, research efforts are being intensified on the use of botanicals as alternatives to commonly used synthetic insecticides because many plants demonstrate insecticidal activities against insect pests and plant products are more biodegradable, and thus pose fewer problems to the environment (Boeke *et al.*, 2004; Isman, 2008; Yeon *et al.*, 2011).

Azadirachta indica A. Juss commonly called neem is toxic to over 500 insect species (Schmutterer, 1990; Athanassiou *et al.,* 2005; Kavallieratos *et al.*, 2007; Roy *et al.*, 2010) including stored product insect pests of cowpea and maize (Bélanger and Musabyinama, 2005; Iloba and Ekrakene, 2006; Debashri and Tamal, 2012). However, research on the insecticide properties of *A. indica* is scanty in some African countries. The efficacy of Cameroonian *A. indica* seed powder and oil against *S. zeamais*, and only the seed powder against *C. maculatus* has been reported (Nukenine *et al.,* 2011a, 2011b, 2013, Tofel *et al.*, 2015). More so, during harvesting or drying *A. indica* seeds may be contaminated with aflatoxins (Kausik *et al.*, 2002). In developed countries, where regulations and facilities about the safety control of plant products exist, it is easy to minimize the risk of *A. indica* seed contaminations. In these countries, drying neem seed is therefore not a problem because equipment like oven which could be used to dry the seeds safely is present. This is not the case in developing countries where *A. indica* is wide-spread and fast-drying could mainly be achieved through sun-drying. However, many researchers have contended that sun-drying causes photo- and thermo-degradation of plant materials including *A. indica* (Johnson *et al.*, 2000; Najafian and Agah, 2012; Shahhoseini *et al.*, 2013), which leads to a significant reduction in their bio-activity against pests and in humans. Thus, subsistence farmers and traditional doctors are advised to dry their plant materials in shade before mixing with grains in storage and use as medication, respectively, for better efficacy. Unfortunately, shade-drying of *A. indica* seeds may encourage the proliferation of fungi and the production of aflatoxins in these products, which would in turn attain humans causing serious health hazards.

To promote the use of safer *A. indica* seed oil combined with good efficacy in crop protection including stored products, the mode of drying of the seeds needs to be re-examined.

The cowpea weevil *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae) and the maize weevil *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) are widely distributed in tropical regions. They are known respectively as serious insect pests of legumes and cereals in storage. *C. maculatus* is responsible for about 30 to 60% weight losses of stored cowpea within six months (Adedire and Ajayi, 2003; Ketoh *et al.*, 2005) while 30 to 40% maize weight losses are common with *S. zeamais* infestations (Parugrug and Roxas, 2008; Yuya *et al.*, 2009). About 75% of harvested cowpea and maize are stored by farmers in Africa (Kumar, 1991). To reduce post-harvest losses, different methods of grain protection are used by small holder farmers as well as at the industrial level (Isman, 2006).

Therefore, the present study was proposed to extend the bio-efficacy assay of Cameroonian *A. indica* seed oil to *C. maculatus* and determine the Azadirachtin A and fatty acid contents as well as the insecticidal efficacy of oils extracted from *A. indica* seeds and kernels that were dried in the shade or in sun-light against *S. zeamais* on maize and *C. maculatus* on cowpea. Adult mortality, reduction of progeny emergence and reduction of grain damage were the parameters used to assess the insecticidal efficacy of the *A. indica* oils.

Materials and methods

Insects

Sitophilus zeamais was reared on maize and *C. maculatus* on cowpea in controlled temperature and humidity chambers (25 ± 1 °C and 60 ± 3% r.h.) in darkness. Adults of *S. zeamais* and *C. maculatus* were obtained from laboratory colony kept since 1968 and 2011, respectively at the Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection, Julius Kühn-Institute (JKI), Berlin, Germany. Insects aged 1 day for *C. maculatus* and between 7-14 days for *S. zeamais* were used for all bioassays with cowpea and maize as substrates, respectively. The maize variety was yellow Ricardino (KWS) harvested in an experimental field of JKI, Braunschweig, Germany in 2012. The organic cowpea (Black-eyed bean, Perou variety) was purchased in a tropical food store in Berlin, Germany.

Collection of Azadirachta indica seeds and extraction of the oil

Ripe seeds (de-pulped by birds) were collected on the ground under *A. indica* trees in the Meskine quarter (latitude 10°33.16' N, longitude 14°815.04' E and altitude of 356 m.a.s.l.) of Maroua, Far-North region, Cameroon in May 2011. The city of Maroua is in the Sudano-Sahelian agro-ecological zone (IRAD, 2007). This agro-ecology is characterized by two seasons: wet (June to September) and dry (October to May). Annual rainfall ranges between 800 and 1000 mm. Annual mean temperature is 29 °C, with a maximum of 39 °C in March and minimum of 17 °C in January. Average annual r.h. stands at 67%.

The collected seeds were subjected to four different drying regimes: kernel in shade (Shade-dried kernel), kernel in sunlight (sun-dried kernel), seeds in shade (Shade-dried undehusked seeds) and seeds in sunlight (sun-dried undehusked seeds). The drying temperatures of the seeds and kernels were 27 ± 3 °C and 34 ± 4 °C in shade and in sunlight, respectively. The dried seeds were dehusked and together with the dried kernels were stored in a deep-freezer at -14 °C, until transported to Berlin, Germany (after 4 months).

The extraction of the oil was carried out using a mechanical press (CA59G Komet, Mönchengladbach, Germany). Two kilogram each of kernels from the four drying regimes were introduced into the press and crude *A. indica* oils were obtained, filtered and weighed for the determination of the yields in oil.

Azadirachtin A content determination

Extraction and cleanup of the *A. indica* seed oils from the different drying regimes were carried out using QuEChERS (Anastassiades, 2003). 100 µl of oil was weighed into a 50 ml polypropylene centrifuge tube and 100 µl of surrogate (Spinosyn A 100 g/l). Extraction was performed by adding 10 ml acetonitrile and 10 ml of water in every tube and each tube was shaken using a vortex-mixer for 45 min and then in an ultrasonic bath for 15 min. To cleanup, anhydrous $MgSO_4$ (4 g) and NaCl (1 g) were added and the tubes were tightly capped and vigorously mixed with vortex for 1 min. The extracts were centrifuged at 3000 g × 5 min. After centrifugation, an aliquot of 100 µl from the upper layer of extract was transferred to a vial and then dried to evaporate water. The extract was diluted with 1 ml of methanol/water 1:1, (v/v) containing an internal standard Spinosyn L (used for quantification) at the concentration of 25 pg/µl and subsequently kept in the dark at 4°C until analyzed by LC/MS/MS. According to drying method each treatment was replicated thrice and for each tube two replications was done for a total of six repetitions.

Liquid chromatography–electrospray ionization-tandem mass spectrometry, in positive ion mode, was used to separate, identify, and quantify azadirachtin A. For the LC analysis, a Shimadzu Prominence UFLCXR HPLC system (Agilent Technologies, Darmstadt, Germany) with a binary pump was used. The analytical column employed was a reversed-phase C18 of 50 × 3 mm and 2.6 µm particle sizes. The mobile phase A was methanol-water (90:10, v/v) with 0.1% acetic acid + 5 mmol Ammonium acetate. The mobile phase B was water with 0.1% acetic acid + 5 mmol Ammonium acetate. The gradient program started with 0% of A, constant for 2 min, followed by

a linear gradient up to 100% A in 3.5 min, and finishing with 100 % A constant for 3.5 min. After this 5.5 min run time, 3.5 min of post-time followed using the initial 30% of B. The flow rate was set constant at 0.9 ml/min during the whole process, and the injection volume was 5 µl. For the mass spectrometric analysis, a AB SCIEX QTRAP 4000 MS/MS system (AB Sciex Instruments) was used, equipped with a turbo ion spray source operating in positive ionization mode, set with the following parameters: Ion Spray (IS) voltage: 5500 V; curtain gas: 20 psi; nebulizer gas (GS1): 70 psi; auxiliary gas (GS2): 50 psi; source temperature: 550 °C. Nitrogen was used as the nebulizer and collision gas. Optimization of the compound was performed by flow injection analysis (FIA), injecting individual standard solutions directly into the source. AB SCIEX Analyst software 1.5.2 was used for data acquisition and processing.

Fatty acid determination

Crude oils were analyzed as methyl esters to determine the fatty acid composition. Fatty acid methyl esters (FAME) were obtained through a two-step method with sodium methoxyde and HCl as catalysts, and then analyzed by capillary column gas chromatography (GC) (Hewlett Packard HP 6890) equipped with a flame-ionization detector (FID), as described in EN ISO 5509 and EN ISO 5508. 1 ml of the FAME sample was injected and GC separation was carried out in a HP-INNO Wax capillary column (Hewlett Packard; 30 m length, 0.25 mm i.d. and 0.25 m film thickness).

Adult toxicity test and F_1 progeny production

The volumes of 0.1, 0.15, 0.2, 0.25 and 3 ml of *A. indica* oils from sun-dried seeds, sun-dried kernels, shade-dried seeds and shade-dried kernels were separately pipetted to 50 g of maize or cowpea in 250 ml glass jars to give the concentrations of 2, 3, 4, 5 and 6 ml/kg of maize or cowpea. Controls consisted of grains without neem seed oil. Each jar was shaken with a bidimensional mixer (Gerhardt, Dreieich, Germany) for approximately 4 min to ensure uniform distribution of the oils to the entire grain mass. Groups of 20 *S. zeamais* and *C. maculatus* were separately added to glass jars containing the treated maize and cowpea, respectively. Control gas jars also separately received twenty insects each. All treatments were arranged in a completely randomized design on shelves in the laboratory (25 ± 1 °C and 60 ± 3% r.h.) and each treatment had four replications. Mortality was recorded 1, 3, 7 and 14 days after treatment for *S. zeamais* and 1, 3 and 6 days after treatment for *C. maculatus*. Insects were considered dead when no movement was observed after touching them carefully with entomological forceps. After the 14-day and 6-day mortality recordings respectively for *S. zeamais* and *C. maculatus*, all the insects were separated from the grains and discarded. The grains were left inside the jars and all F_1 progeny were counted (Nukenine *et al.*, 2007).

Damage on grains

Similar dosages of each type of *A. indica* oil, as for the toxicity bioassay described above, were considered for100 g grains. A group of 30 adult insects of mixed sex were introduced into each jar containing treated or untreated grains. All treatments were replicated four times. After 10 weeks of storage, one hundred grains were randomly selected from each treatment of maize and cowpea (Udo 2005) and the number of damaged grains (grains with characteristic holes) and undamaged grains were counted and weighed. Percent weight loss (PWL) was computed using FAO (1985) method as follows:

$$PWL = [(U \times Na) - (Ua \times Ne)] / U\,(Na + Ne) \times 100$$

Where U is the weight of undamaged fraction in the sample, U*a* is the weight of the damaged fraction, N*a* is the number of damaged grains in the sample; N*e* is the number of undamaged grain in the sample.

The Percentage grain damage (PD) was therefore, calculated using the formula: PD =B/A × 100

Where: B is number of grains with holes and A is the total number of grains.

Data analysis

The mortality counts were corrected with Abbott's

(1925) formula. Data on % cumulative corrected mortality, % reduction of progeny production, % grain damage and % weight loss were transformed to the arcsine [(square root(x/100)] and the number of progeny produced was log-transformed, then subjected to the ANOVA procedure of the Statistical Analysis System (SAS Version 9.2). Tukey's (HSD) mean separation test was employed with a significance of 95% (P = 0.05). The concentration required to kill 50% of insects (LC_{50}) was estimated using probit analysis (Finney, 1971).

Results

Yield and Azadirachtin and fatty acid contents of Azadirachta indica oils from seeds exposed to different drying regimes

The yield of the oils from *A. indica* seeds that were subjected to the four drying regimes ranged from 28.30% (sun-dried seeds) to 34.42% (shade-dried kernels), with sun-dried seeds/kernels tending to produce lower quantities of oils than the shade-dried seeds/kernels (Table 1).

Table 1. Azadirachtin A content and oil yields of *Azadirachta indica* seeds that were subjected to different drying regimes.

Dryingregime	Yield (% w/w)	Azadirachtin† (g/kg)
Shade-dried kernels	34.42	3.56 ± 0.14[a]
Sun-dried kernels	28.60	3.09 ± 0.09 [ab]
Shade-dried seeds	32.70	3.69 ± 0.16 [a]
Sun-dried seeds	30.30	2.89 ± 0.17 [b]
F (3, 8) ‡		7.06*

† Means (± SE) in the same line followed by the same letter do not differ significantly (Tukey's test; P < 0.05)

‡ * P < 0.05.

The oil from the sun-dried seeds had lower Azadirachtin contents compared with the oils from the other three drying regimes (shade-dried seeds, sun-dried kernels and shade-dried kernels), which had similar contents of the substance.

The major fatty acids found in the *A. indica* seed oils in decreasing order were oleic acid >> linoleic, palmitic and stearic acids >>> Arachidic, behenic and lignoceric acids, regardless of drying regime (Table 2). However, the contents of all the fatty acids were similar among the oils of the seeds subjected to the four drying regimes.

Table 2. Fatty acid composition of *Azadirachta indica* crude oils from seeds that were subjected to different drying regimes.

Fatty acid (%)	Drying regime†				
	Shade-dried kernels	Sun-dried kernels	Shade-dried seeds	Sun-dried seeds	F (3, 12) ‡
Palmitic acid	16.00 ± 0.02[c]	15.86 ± 0.04[c]	16.84 ± 0.75[b]	16.41 ± 0.02[b]	1.37 ns
Linoleic acid	16.66 ± 0.03[b]	16.75 ± 0.07[b]	12.21 ± 0.07[b]	16.38 ± 0.08[b]	1.08 ns
Oleic acid	50.03 ± 0.06[a]	51.55 ± 0.25[a]	53.67 ± 0.49[a]	51.82 ± 0.11[a]	1.42 ns
Stearic acid	15.45 ± 0.09[d]	14.48 ± 0.16[d]	15.32 ± 0.66[b]	14.05 ± 0.09[c]	1.07 ns
Arachidic acid	1.53 ± 0.04[e]	1.11± 0.07[e]	1.44 ± 0.07[c]	1.37 ± 0.01[d]	0.84 ns
Behenic acid	0.22 ± 0.07[f]	0.14 ± 0.08[f]	0.30 ± 0.01[c]	0.13 ± 0.08[e]	1.03 ns
Lignoceric acid	0.06± 0.06[f]	0.11± 0.07[f]	0.13 ± 0.08[c]	0.06 ± 0.06[e]	0.33 ns
F (6, 21) ‡	90544.2***	9522.00***	104.23***	60632.9***	

† Means (± SE) in the same line followed by the same letter do not differ significantly (Tukey's test; P < 0.05)

‡ ns P > 0.05; *** P < 0.001.

Toxicity tests

All the *A. indica* seed oils generally caused significant mortality to adult *C. maculatus* and *S. zeamais* (Tables 3 and 4) compared to the control. Overall, no significant difference was observed among the oils derived from seeds that were subjected to the four drying regimes regarding the mortality they caused to *S. zeamais* and *C. maculatus*. Percentage mortality increased with increase of dose levels for the two insect species, but the rate of increase in mortality with days after exposure was lower for *C. maculatus* (Table 3) compared to *S. zeamais* (Table 4).

Table 3. Corrected cumulative mortality of adult *Callosobruchus maculatus* exposed in cowpea grains treated with *Azadirachta indica* oils extracted from seeds that were subjected to different drying regimes.

Exposure period (days)	Dose (ml/kg)	Drying regime / % Mortality (mean ± SE) †				
		Shade-dried kernels	Sun-dried kernels	Shade-dried seeds	Sun-dried seeds	$F_{(3, 12)}$ ‡
1	0	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	
	2	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	–
	3	7.50 ± 7.50^{bc}	11.75 ± 3.13^{c}	13.75 ± 5.15^{b}	2.50 ± 1.44^{d}	1.90 ns
	4	25.00 ± 12.08^{b}	15.00 ± 2.89^{c}	32.50 ± 11.27^{b}	21.25 ± 6.57^{c}	0.53 ns
	5	60.00 ± 5.77^{a}	57.50 ± 9.68^{b}	65.00 ± 5.00^{a}	53.75 ± 3.75^{b}	0.49 ns
	6	77.50 ± 1.44^{a}	83.75 ± 5.54^{a}	81.25 ± 2.39^{a}	76.25 ± 4.37^{a}	0.84 ns
	$F_{(5, 18)}$ ‡	27.64***	50.42***	32.37***	79.92***	
3	0	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0,00^{e}	0.00 ± 0.00^{c}	
	2	3.75 ± 2.39^{d}	3.75 ± 2.39^{e}	1.25 ± 1.25^{e}	0.00 ± 0.00^{c}	1.58 ns
	3	13.75 ± 6.57^{cd}	21.25 ± 4.73^{d}	13.82 ± 4.23^{d}	6.25 ± 3.15^{c}	1.09 ns
	4	37.50 ± 12.67^{bcAB}	50.86 ± 10.65^{cA}	33.75 ± 1.25^{cB}	46.25 ± 9.87^{bA}	2.65*
	5	76.25 ± 5.15^{ab}	83.75 ± 3.75^{b}	83.49 ± 2.54^{b}	71.25 ± 7.74^{b}	1.34 ns
	6	90.00 ± 4.08^{aB}	100.00 ± 0.00^{aA}	100.00 ± 0.00^{aA}	100.00 ± 0.00^{aA}	7.95*
	$F_{(5, 18)}$ ‡	34.99***	175.51***	80.81***	63.96***	
6	0	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	
	2	10.40 ± 2.15^{cB}	21.45 ± 2.22^{cA}	6.45 ± 2.45^{cdB}	3.88 ± 2.51^{deB}	5.79*
	3	23.42 ± 5.53^{bc}	38.95 ± 10.13^{bc}	28.73 ± 8.48^{c}	13.11 ± 3.32^{d}	2.22 ns
	4	41.84 ± 12.86^{b}	63.36 ± 5.41^{b}	73.54 ± 12.47^{b}	57.41 ± 11.87^{c}	1.54 ns
	5	87.11 ± 3.20^{aB}	96.05 ± 3.95^{aA}	88.14 ± 2.55^{abB}	86.86 ± 6.25^{bB}	2.78*
	6	98.69 ± 1.32^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	1.00 ns
	$F_{(5, 18)}$ ‡	47.10***	41.35***	46.76***	58.71***	

† Means in the same column followed by the same lowercase letter within the same exposure period or in the same line followed by the same uppercase letter, do not differ significantly (Tukey's test; $P < 0.05$)

‡ ns $P > 0.05$; * $P < 0.05$; *** $P < 0.001$; – F value estimation is not possible due to equal variance.

Generally, drying regime had no effect on the mortality of the two insect species caused by the *A. indica* seed oils. Nonetheless, the sun-drying of seeds and kernels led to a higher mortality of *C. maculatus,* 3 (4 and 6 ml/kg) and 6 (2 and 5 ml/kg) days post exposure. The oil from the sun-dried kernels of *A. indica* caused greater mortality to *S. zeamais* than that from the shade-dried kernel only 7 days after treatment for the 4 ml/kg dose level. The highest tested dose (6 ml/kg) of *A. indica* oil achieved complete mortality of *C. maculatus* 3 days post exposure for all the drying regimes, except the shade-dried kernels which caused a maximum mortality of 98.69%, six days after exposure. Oils from the sun-

dried kernels and seeds caused total mortality to *S. zeamais* 7 days after exposure with the respective doses of 5 and 6 ml/kg. For the shade dried kernels and seeds, the oil respectively caused a maximum mortality of 98.75% (6 ml/kg) and 100% (5 ml/kg) to the weevil, 14 days after exposure.

Table 4. Corrected cumulative mortality of adult *Sitophilus zeamais* exposed in maize grains treated with *Azadirachta indica* oils extracted from seeds that were subjected to different drying regimes.

Exposure period (days)	Dose (ml/kg)	Drying regime / % Mortality (mean ± SE)				
		Shade-dried kernels	Sun-dried kernels	Shade-dried seeds	Sun-dried seeds	$F_{(3, 12)}$ ‡
1	0	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}	
	2	1.25 ± 1.25^{c}	1.25 ± 1.25^{c}	0.00 ± 0.00^{d}	1.25 ± 1.25^{b}	0.33 ns
	3	5.00 ± 2.04^{bc}	3.75 ± 2.39^{bc}	2.50 ± 1.44^{cd}	5.00 ± 3.54^{ab}	0.19 ns
	4	7.50 ± 3.23^{bc}	7.50 ± 1.44^{bc}	8.75 ± 2.39^{bc}	6.25 ± 2.39^{ab}	0.30 ns
	5	13.75 ± 2.30^{ab}	17.50 ± 2.50^{ab}	11.25 ± 1.25^{ab}	17.50 ± 4.33^{a}	1.20 ns
	6	20.00 ± 4.56^{a}	31.25 ± 6.57^{a}	17.50 ± 2.50^{a}	18.75 ± 4.27^{a}	1.75 ns
$F_{(5, 18)}$ ‡		8.31***	14.87***	19.12 ***	6.92**	
3	0	0.00 ± 0 .00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	
	2	8.75 ± 5.54^{cd}	5.00 ± 2.04^{d}	7.50 ± 1.44^{cd}	8.75 ± 3.75^{de}	0.24 ns
	3	16.25 ± 2.39^{bc}	17.50 ± 6.29^{c}	15.00 ± 2.04^{cd}	16.25 ± 1.25^{cd}	0.10 ns
	4	28.75 ± 7.18^{bc}	37.50 ± 5.95^{bc}	22.50 ± 2.50^{bc}	30.00 ± 5.40^{bc}	1.29 ns
	5	40.00 ± 4.56^{ab}	47.50 ± 1.44^{ab}	40.00 ± 4.08^{ab}	40.00 ± 4.56^{ab}	0.99 ns
	6	86.25 ± 7.74^{a}	62.50 ± 7.22^{a}	56.25 ± 7.47^{a}	51.25 ± 3.15^{a}	0.46 ns
$F_{(5, 18)}$ ‡		15.27***	27.75***	24.91***	30.23 ***	
7	0	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	
	2	23.79 ± 9.44^{c}	26.25 ± 6.88^{c}	26.25 ± 10.68^{cd}	23.75 ± 5.54^{c}	0.03 ns
	3	37.50 ± 6.61^{bc}	65.00 ± 10.61^{b}	50.00 ± 6.12^{bc}	51.25 ± 9.00^{b}	1.79 ns
	4	72.50 ± 8.54^{abB}	91.25 ± 5.15^{aA}	71.25 ± 8.26^{abB}	70.00 ± 3.54^{bB}	3.02 *
	5	95.00 ± 3.54^{a}	100 ± 0.00^{a}	95.00 ± 3.54^{a}	93.75 ± 4.73^{a}	0.85 ns
	6	95.00 ± 2.89^{a}	100 ± 0.00^{a}	96.25 ± 2.39^{a}	100.00 ± 0.00^{a}	1.96 ns
$F_{(5, 18)}$ ‡		41.35***	57.00***	37.46***	58.58*	
14	0	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	
	2	30.40 ± 7.08^{c}	39.15 ± 9.97^{b}	31.84 ± 10.85^{c}	33.78 ± 8.20^{c}	0.17 ns
	3	77.38 ± 1.31^{b}	74.80 ± 9.06^{a}	74.15 ± 7.84^{b}	71.70 ± 6.40^{b}	1.73 ns
	4	98.60 ± 1.40^{a}	95.00 ± 3.54^{a}	88.62 ± 6.57^{ab}	88.41 ± 6.54^{ab}	0.40 ns
	5	97.50 ± 2.50^{a}	100 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	1.00 ns
	6	98.75 ± 1.25^{a}	100 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	1.00 ns
$F_{(5, 18)}$ ‡		66.42***	51.22***	45.49***	65.49***	

† Means in the same column followed by the same lowercase letter within the same exposure period or in the same line followed by the same uppercase letter, do not differ significantly (Tukey's test; $P < 0.05$))

‡ ns $P > 0.05$, * $P < 0.05$; ** $P < 0.01$, *** $P<0.001$; – F value estimation is not possible due to equal variance.

The 3-day LC_{50} values were 3.89 ml/kg (sun-dried kernels), 3.89 ml/kg (shade-dried seeds)), 4.13 ml/kg (shade-dried kernels) and 4.16 ml/kg (sun-dried seeds) for *C. maculatus*, and the 7-day LC_{50} values for *S. zeamais* were 2.53 ml/kg for the sun-dried kernels, 2.83 ml/kg for the shade-dried seeds, 2.86 ml/kg for the sun-dried seeds and 3.00 ml/kg for the shade-dried kernels.

F_1 Progeny production

In all the evaluated treatments, the application of *A. indica* seed oils completely suppressed F_1 progeny

emergence in *C. maculatus*, regardless of the drying regime to which the seeds where subjected (Table 5). Except for maize treated with the lowest dose 2 ml/kg with Sun- and shade-dried kernels and sun-dried seeds, all dose levels of the oils from the seeds dried under the four regimes caused 100% reduction in *S. zeamais* F_1 progeny emergency (Table 6). The oils from the seeds tended to reduce F_1 progeny emergence in the weevil than those from the kernels, when maize seeds were treated with the lowest dose 2 ml/kg.

Table 5. Progeny production of *Callosobruchus maculatus* in cowpea grains treated with *Azadirachta indica* oils extracted from seeds that were subjected to different drying regimes.

	Drying regime				
Dose (ml/kg)	Shade-dried kernels	Sun-dried kernels	Shade-dried seeds	Sun-dried seeds	$F_{(3, 12)}$ ‡
	Number (mean ± SE) of F_1 adult progeny †				
0	436.50 ± 22.91[a]	432.25 ± 11.84[a]	460.75 ± 24.08[a]	473.75 ± 20.17[a]	0.94 ns
2	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	–
3	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	–
4	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	–
5	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	–
6	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	–
$F_{(5, 18)}$ ‡	362.98***	1332.39***	366.19***	551.81***	
	Percentage (mean ± SE) reduction in adult emergence relative to control †				
0	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	–
2	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	–
3	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	–
4	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	–
5	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	–
6	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	–
$F_{(5, 18)}$ ‡	–***	–***	–***	–***	

† Means in the same column followed by the same letter do not differ significantly (Tukey's test; $P < 0.05$)

‡ ns $P > 0.05$; *** $P < 0.001$; – F value estimation is not possible due to equal variance.

Damage on grains

The infested cowpea (*C. maculatus*) and maize (*S. zeamais*) grains that were previously treated with *A. indica* oils extracted from seeds that were subjected to the four drying regimes had no damaged grains and recorded no weight loss, ten weeks after infestation, when the dose level was ≥ 3 ml/kg (Tables 7 and 8). When treated with 2 ml/kg of the *A. indica* seed oils, both cowpea and maize grains recorded very little damage and weight losses compared to the control, although the value for these parameters were higher for maize (2.25 – 4.50% damage and 0.33 – 0.75 % weight loss) than cowpea (0.00 – 0.75% grain damage and 0.00 – 0.06% weight loss). For this dosage level, the damage caused by *C. maculatus* to cowpea seeds and *S. zeamais* to maize seeds, as well as the resulting weight losses, were similar across the four drying regimes.

Discussion

The results of *A. indica* oil yields in the present study showed that sun-dried kernels produced lower quantity of oil (28.60% w/w) than the other drying regimes. Faye (2010) reported that dehusked neem seeds (kernels) gave lower oil quantity than undehusked seeds. In the same line, Soetaredjo *et al.* (2008) observed that when the exposure temperature of neem seeds increased, the yield of oil decreased

from 32% at room temperature to 18% at 80°C. They noticed that drying seeds in sunlight reduces their moisture contents and leads to the attachment of the oil to the proteins within the seed structures. Kumar and Parmar (1996), Munoz-Valenzuela *et al.* (2007) and Jadega *et al.* (2011), screened *A. indica* seeds from different regions in India and Mexico and found that the yield of the oil ranged from 15.4 to 54%, the range of 28.60% to 34.42% for the present study is in accordance with their findings. These authors found that variation in yield of the oil was independent on the age of the trees and the origin of the seeds but dependent on rainfall, humidity and temperature of the area.

Table 6. Progeny production of *Sitophilus zeamais* in maize grains treated with *Azadirachta indica* oils extracted from seeds that were subjected to different drying regimes.

Dose (ml/kg)	Drying regime				
	Shade-dried kernels	Sun-dried kernels	Shade-dried seeds	Sun-dried seeds	$F_{(3, 12)}$ ‡
	Number (mean ± SE) of F_1 adult progeny †				
0	48.50 ± 7.35^{a}	46.50 ± 8.87^{a}	44.50 ± 3.69^{a}	42.50 ± 2.33^{a}	0.14 ns
2	7.25 ± 0.48^{bA}	5.25 ± 1.93^{bA}	0.00 ± 0.00^{bB}	3.25 ± 2.29^{bAB}	6.32 **
3	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
4	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
5	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
6	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
$F_{(5, 18)}$ ‡	41.69***	54.28***	61.09***	99.01***	
	Percentage (mean ± SE) reduction in adult emergence relative to control †				
0	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	
2	84.28 ± 2.15^{bB}	88.71 ± 4.10^{bAB}	100.00 ± 0.00^{bA}	92.75 ± 4.94^{bA}	7.73 **
3	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	–
4	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	–
5	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	–
6	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	–
$F_{(5, 18)}$ ‡	2087.73***	2143.36***	–***	230.11***	

† Means in the same column followed by the same lower case letter or in the same line followed by the same upper case letter, do not differ significantly (Tukey's test; $P < 0.05$).

‡ ns $P > 0.05$, ** $P < 0.01$, *** $P < 0.001$; – F value estimation is not possible due to equal variance.

Unsaturated fatty acids (oleic acid and linoleic acid) were higher (68%) than saturated fatty acids (palmitic acid, stearic acid, arachidic acid behenic acid and lignoceric acid) in our *A. indica* seeds for all the four drying regimes. The presence of unsaturated fatty acids in *A. indica* seed oil is an important indicator of the quality of the oil (Kaushik, 2002) and it reduces the degradation rate of azadirachtin A (Johnson *et al.*, 2000), which is the main compound in *A. indica* oil reputed for insecticidal efficiency. Kaushik and Vir (2000), Djenontin *et al.* (2012) and Tomar *et al.* (2012), recorded similar results to that of the present study, with respect to the type of fatty acids and the patterns of the saturated and unsaturated fatty acids found in *A. indica* seed oils from India and Nigeria. Also, the diversity and quantity of the fatty acids in this study are close to those obtained with the edible oils of the oleic type such as that extracted from groundnut (Kapseu and Parmentier, 1999).

It is widely reported that the sun-drying of plant materials has an effect on their chemical composition and therefore reduced their efficacy when used as medications or insecticides (Caboni *et al.* 2009; Najafian and Agah, 2012; Shahhoseini *et al.*, 2013). Johnson *et al.* (2003), Rembold (2004) reported that

Azadirachtin is extremely labile in light with photolysis half live ranging from 48 min to 3.98 days in thin films, under UV light. The Azadirachtin A content in the oil obtained from the sun-dried seeds in the present study was less compared to other drying regimes. Sidhu *et al.* (2003) studied the variation of Azadirachtin A of *A. indica* oil of 43 provenances in India. They recorded a range from 0.55 to 3.03 g/kg of Azadirachtin A, with only those from four provenances reaching the rate 2.00 g/kg, thus even the sun-dried kernels and sun-dried seeds oil in the present study had higher Azadirachtin A contents compared to theirs. This difference in Azadirachtin content may be explained by the variation of the geographical locations (Ermel *et al.*, 1986). Soils and climate may influence the Azadirachtin A contents in plants (Sidhu *et al.*, 2003; Gupta *et al.*, 2010).

Table 7. Grain damage and weight loss of cowpea caused by *Callosobruchus maculatus* in grains treated with *Azadirachta indica* oils extracted from seeds that were subjected to different drying regimes and then stored for 10 weeks.

Damage and doses (ml/kg)	Drying regime				
	Shade-dried kernels	Sun-dried kernels	Shade-dried seeds	Sun-dried seeds	$F_{(3, 12)}$ ‡
	Mean (± SE) grain damage (%) †				
0	97.25 ± 0.75^{a}	97.25 ± 0.48^{a}	97.00 ± 0.41^{a}	97.25 ± 0.48^{a}	0.35 ns
2	0.25 ± 0.25^{b}	0.00 ± 0.00^{b}	0.75 ± 0.00^{b}	0.25 ± 0.25^{b}	0.41 ns
3	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
4	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
5	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
6	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
$F_{(5, 18)}$ ‡	1594.95***	466.90***	946.09***	2153.73***	
0	28.52 ± 1.19^{aB}	39.68 ± 1.84^{aA}	36.29 ± 2.59^{aAB}	42.86 ± 2.80^{aA}	8.07 **
2	0.04 ± 0.04^{b}	0.00 ± 0.00^{b}	0.06 ± 0.06^{b}	0.01 ± 0.01^{b}	0.45 ns
3	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
4	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
5	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
6	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
$F_{(5, 18)}$ ‡	570.00***	95.97***	196.36***	234.48***	

† Means in the same column followed by the same lower case letter or in the same line followed by the same upper case letter, do not differ significantly (Tukey's test; $P < 0.05$).

‡ ns $P > 0.05$, ** $P < 0.01$, *** $P < 0.001$; – F value estimation of is not possible due to equal variance.

The increase in adult mortality of *C. maculatus* and *S. zeamais* with increasing dose and time post exposure, irrespective of the drying regime suggests that the toxicity of the botanicals to the insects depends on the quantity of the active ingredients, which were not generally related to the drying regime. Mbaiguinam *et al.* (2006) obtained 100% mortality of *C. maculatus* with 5 ml/kg of *A. indica* seed oils from Chad, while Wadehi *et al.* (2013) reported that *A. indica* seed oil from Egypt at the same rate caused 100% mortality to *S. zeamais*. The complete mortality of *C. maculatus* and *S. zeamais* achieved in our study when cowpea and maize were treated with *A. indica* seed oil from the sun-dried kernels (6 ml/kg) within 3 and 7 days after exposure, respectively is similar to those of the previous authors. However, Obeng-Ofori and Amiteye (2005) obtained better efficacy with groundnut and soybean oil from Ghana at the rate of 5 g/kg, which caused 93% mortality to *S. zeamais* within 24 h of exposure. This difference in results for *S. zeamais* mortality among the vegetable oils may highlight the fact that *A. indica* oil as opposed to other vegetable oils has antifeedant properties, caused by its limonoids constituents like azadirachtin, nimbin, salanin, nimbidin and meliantriol (Schumutterer, 1990; Addea-Mensah, 1998). Antifeedancy leads to a

slower rate in mortality. Azadirachtin activates deterrent cells in the chemoreceptors of the mouthparts, interferes with other taste chemoreceptors, and blocks firing of "sugar" receptor cells which are responsible for stimulating feeding. These combined effects may result in death by anorexia (primary antifeedancy) (Rukmini, 1987; Schmutterer, 1990; Petit, 2008; Anuradha and Annadurai, 2008). The limonoid compounds also inhibit peristalsis, reduces the production of digestive enzymes as food moves through the gut, restrain mid-gut cell replacement and food intake (secondary antifeedancy) (Mordue and Blackwell, 1993; Koul *et al.*, 2004; Pamela, 2009).

Table 8. Grain damage and weight loss of maize caused by *Sitophilus zeamais* in grains treated with *Azadirachta indica* oils extracted from seeds that were subjected to different drying regimes and then stored for 10 weeks.

Damage and doses (ml/kg)	Drying regime †				
	Shade-dried kernels	Sun-dried kernels	Shade-dried seeds	Sun-dried seeds	$F_{(3, 12)}$ ‡
	Mean (± SE) grain damage (%) †				
0	50.00 ± 1.73^{aA}	45.50 ± 2.84^{aA}	39.75 ± 3.90^{aAB}	37.75 ± 1.93^{aB}	4.13 *
2	3.25 ± 1.25^{b}	4.50 ± 0.29^{b}	2.25 ± 1.03^{b}	2.25 ± 1.44^{b}	1.30 ns
3	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	–
4	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	–
5	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	–
6	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	–
$F_{(5, 18)}$ ‡	458.91***	245.95***	95.10***	97.35***	
	Mean (± SE) weight loss (%) †				
0	17.12± 2.75^{aA}	10.05 ± 0.75^{aB}	12.09 ± 1.50^{aAB}	10.77 ± 1.10^{aB}	3.58 *
2	0.56 ± 0.16^{b}	0.75 ± 0.21^{b}	0.61 ± 0.29^{b}	0.33 ± 0.21^{b}	0.93 ns
3	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
4	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
5	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
6	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
$F_{(5, 18)}$ ‡	38.21***	163.20***	61.78***	91.62***	

† Means in the same column followed by the same lower case letter or in the same line followed by the same uppercase letter do not differ significantly (Tukey's test; $P < 0.05$).

‡ ns $P > 0.05$, * $P < 0.05$, *** $P < 0.001$; – F value estimation of is not possible due to equal variance.

Like all bruchids, adult *C. maculatus* does not feed, while adult *S. zeamais* feeds on maize grains, but the *A. indica* seed oil caused greater mortality to *C. maculatus* than *S. zeamais,* and this was remarkable from the first day after infestation. Vegetable oils are known to penetrate the cuticle of insects (Ibrahim *et al.,* 1999) and also block the spiracles, which will in turn prevent respiration, leading to the death of the insect by asphysiation (Don-Pedro, 1989; Iloba and Ekrakene, 2006). The sclerotization of insect cuticles increases with age and the cuticle becomes hardened and darkened, as a result of additional wax layers, leading to less permeability with age (Odeyemi *et al.*, 2010). The 1-d old *C. maculatus* were much younger than the 7 to 14-d old *S. zeamais* in the present study. The elytras of *C. maculatus* partially covers the dorsal abdomen, while with *S. zeamais* the dorsal abdomen is completely covered by the elytras. More so, *C. maculatus* is more mobile than *S. zeamais,* which could lead to a greater contact of the oil with the former than the latter. Therefore, because of the preceding reasons, more *A. indica* oil may have

penetrated the body of *C. maculatus* than *S. zeamais* and the blocking of spiracles would have been more evident with *C. maculatus*, which could explain the higher susceptibility of *C. maculatus* than *S. zeamais* to the *A. indica* seed oils.

The similarity in the insecticidal effectiveness of the oils from the sun-dried kernels and seeds, as well as the shade-dried kernels and seeds against *C. maculatus* and *S. zeamais* is at variance with the findings of Radwan and El-Shiekh (2012) where *A. indica* oil from seeds that were exposed to sunlight compared to those indoors, caused less mortality to the cotton leafworm, *Spodoptera littoralis* Boisduval. (Lepidoptera: Noctuidae). The similarity in the fatty acid composition among the oils from seeds that were subjected to the different drying regimes in the present study could explain why sun-drying had no influence on insecticidal efficacy. Lienard *et al.* (1993) reported that oils with higher contents of fatty acids are more toxic to insects than those with lower levels of the acids. Notwithstanding, further studies are needed to clarify the relationship among fatty acids, limonoid componds and insecticidal efficacy of *A. indica* seed oil (Gauvin *et al.*, 2004).

One of the basic characteristics of an effective grain protectant is its ability to reduce progeny production in treated grains (Khoshnoud *et al.*, 2008). Results of inhibition of progeny production showed that oils extracted from *A. indica* seeds that were subjected to the four drying regimes completely inhibited progeny emergence of *C. maculatus and S. zeamais,* showing their enormous ability to control both insects. The *A. indica* oils might have acted physically or chemically on eggs or immature stages, depending on the insect species. Suppression of emergence in *C. maculatus* could be related to physical action of the *A. indica* seed oil. The coating of the seeds by *A. indica* oil may prevent the eggs from adhering unto the seeds. Therefore, it was not possible for the eggs to hatch in the grains and death ensues. Similar explanations were advanced by other researchers, where *A. indica* seed oil completely inhibited the progeny production of *S. oryzae* and *C. maculatus* (Bamaiyi *et al.*, 2007; Kemabonta and Falodu, 2013; Ilesanmi and Gundula, 2013). In addition, *A. indica* oil, like other vegetable oils, penetrates the chorion of bruchid eggs via the micropyle and the oil might occlude the egg funnel, which blocks exchange with the outside, leading to the asphyxiation of the developing insect, then death follows (Copping and Menn, 2000).

Azadirachta indica seed oils could also inhibit progeny production by non-mechanical mechanisms, especially with *S. zeamais*. Female maize weevil lays eggs inside the grain. If, on treated grains oviposition is not deterred by the presence of the oil, then the development of immature stages could be affect chemically. As the oil has the ability to infiltrate the grains, the larvae of *S. zeamais,* which feed inside the grain would ingest some quantity of azadirachtin and other compounds like nimbin and salanin in *A. indica* oil. These compounds have growth regulatory effects on larvae, such as, disruption of moulting, growth inhibition, malformation, which may block the developmental stages of the weevils or cause mortality of immature stages (Isman, 2006). Udo (2005) stated that, there is a relationship between F_1 progeny emergence and adult mortality. His statement is confirmed by the report of Fekalu *et al.*, (2012) who found that *Gossypium hirsitum* and *Brassica carinata* seed oils reduced adult emergence of *S. zeamais*. But it was not the case in the present work, since there were living *S. zeamais* 14 days (5 ml/kg) after infestation and offspring were recorded at the dosage level of 3 ml/kg.

Cowpea and maize suffer heavy damage and losses during storage due to *C. maculatus* and *S. zeamais,* respectively. In the control treatment, within 10 weeks of storage, 98% and 45% of cowpea and maize, respectively, were damaged. *A. indica* oil protected well maize and cowpea from the damage and the consequent weight loss caused respectively by *S. zeamais* and *C. maculatus*. Adult mortality and the inhibition of progeny emergence must, at least in parts, be responsible for the little or no damage on the commodities. *A. indica* seed oil and *Moringa* seed oil protected cowpea for 60 days without damage

(Ilesanmi and Gundula, 2013). Cashew kernel oil offered 100% protection of maize grains against *S. zeamais* after 90 days (Adedire *et al*., 2012). Niber (1995) concluded that the action of *A. indica* oil to reduce seed damage was chemical rather physical. Ogemah (2003) observed also reduced seed damage on *A. indica* seed oil treated maize against *Prostephanus truncatus* Horn.

Sun-drying compared to shade-drying of *A. indica* seeds and kernels had no significant negative effect on the bio-efficacy of the oil against *C. maculatus* and *S. zeamais* on cowpea and maize grains, respectively. All the oils obtained from *A. indica* seeds following the different drying regimes greatly protected the grains against the infestation of their respective pest insect, although, the oil from the sun-dried kernels tended to show slightly higher bioactivity against the two insects. Because *A. indica* grows in countries where high temperatures around 40°C are common, sun-drying of the seeds could be a suitable method for farmers to obtain safer and cheaper botanicals for the protection of cowpea and maize grains against insect attacks during storage.

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References

Abbott WS. 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology **18,** 265-267.

Addea-Mensah I. 1998. The uses of neem (*Azadirachta indica*) in Ghana and their relations of the chemicals constituents and biological activities. Proc. Semimar held Dodowa: the potentials of the neem trees in Ghana. 11-26 P.

Adedire CO, Ajayi OE. 2003. Potential of sandbox, *Hura crepitans* L. seed oil for protection of cowpea seeds from *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae) infestation. Journal of Plant Diseases and Protection **110,** 602-610.

Adedire CO, Akinkurolere RO, Obembe OM. 2012. Efficacy of cashew kernel extracts in the control of the maize weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae). Archives of Phytopathology and Plant Protection **45,** 831-839. http://dx.doi.org/10.1080/03235408.2011.598259.

Anastassiades M, Lehotay SJ, Štajnbaher D, Schenck FJ. 2003. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. Journal of AOAC International **86,** 412-431.

Anuradha A, Annadurai RS. 2008. Biochemical and molecular evidence of azadirachtin binding to insect actins. Current Science **95 (11),** 1588–1593.

Athanassiou CG, Kontodimas DC, Kavallieratos NG, Anagnou- Veroniki M. 2005. Insecticidal effect of NeemAzal against three stored-product beetle species on rye and oats. Journal of Economic Entomology **98,** 1499-1505. http://dx.**doi**.org/10.1093/jee/98.5.1733 1733-1738.

Bamaiyi LJ, Ndams IS, Toro WA, Odekina S. 2006. Effect of mahogany *Khaya senegalensis* seed oil in the control of *Callosobruchus maculatus* on stored cowpea. Plant Protection Science **42,** 130-134.

Bélanger A, Musabyinama. 2005. Le Neem contre les insectes et maladies. Journées horticoles 2005. 4 p.

Boeke SJ, Baumgart IR, van Loon JJA, van Huis A, Dicke M, Kossou DK. 2004. Toxicity and repellence of African plants traditionally used for the protection of stored cowpea against *Callosobruchus*

maculatus. Journal of Stored Product Research **40,** 423 – 438. http://dx.doi.org/10.1016/S0022-474X(03)00046-8.

Caboni PL, Sarais G, Angioni A, Lai F, Dedola F, Cabras P. 2009. Fate of azadirachtin A and related azadirachtoids on tomatoes after greenhouse treatment. Journal of Environmental Science and Health **44,** 598-605. http://dx.doi.org/10.1080/10934520902784641.

Copping GL, Menn JJ. 2000. Biopesticides: a review of their actions, applications and efficacy. Pest Management Science **56,** 651-676. http://dx.doi.org/10.1002/15264998(200008)56:8<651::AID-PS201>3.0.CO;2-U.

Debashri M, Tamal M. 2012. A review on efficacy of *Azadirachta indica* A. Juss based biopesticides: An Indian perspective. Research Journal in Recent Science **1,** 94-99.

Djenontin TS, Wotto VD, Avlessi F, Lozano P, Sohounhoulé DKC, Pioch D. 2012. Composition of *Azadirachta indica* and *Carapa procera* (Meliaceae) seed oil and cakes obtained after oil extraction. Industrial Crops Production **38,** 39- 45. http://dx.**doi**.org/10.1016/j.indcrop.2012.01.005.

Don-Pedro KN. 1989. Mechanisms of action of some vegetable oils against *Sitophilus zeamais* Motsch (Coleoptera: Curculionidae) on wheat. Journal of stored product Research **25,** 217-223.

Ermel KE, Pahlich E, Schmutterer H. 1986. Azadirachtin content of neem kernels from different geographical locations, and its dependence on temperature, relative humidity and light. Proceeding of the 3rd International. Neem Conference Nairobi, Kenya. pp.171-184.

FAO. 1985. Prevention of post-harvest food losses. Training series N°10. Rome, Food and Agricultural Organization of the United Nations.

Faye M. 2010. Nouveau procédé de fractionnement de la graine de neem (*Azadirachta indica* A. Juss) Sénégalais : Production d'un biopesticide d'huile et de tourteau. PhD Thesis, University of Toulouse, 267 p.

Felaku G, Waktole S, Santiago DR. 2012. Evaluation of plant powders and cooking oils against maize weevil, *Sitophilus zeamais* M. (Coleoptera: Curculionidae) under laboratory conditions. Molecular Entomology **3,** 4-14.

Finney DJ. 1971. Probit analysis. Cambridge University Press, London, United Kingdom.

Gauvin MJ, Bélanger A, Nébié R, Boivin G. 2003. *Azadirachta indica* : l'azadirachtine est-elle le seul ingrédient actif ?». Phytoprotection **84,** 115-119.

Guo H, Yang M, Qi Q. 2014. Insecticidal and antifeedant effects of two alkaloids from *Cynanchum komarovii* against larvae of *Plutella xylostella* L. Journal of Applied Entomology **238,** 133-140. http://dx.doi.org/10.1111/jen.12075.

Gupta VK, Ahlawat SP, Kumar RV, Ajit, Datta A. 2010. Effect of season and year on Azadirachtin A and oil content in neem (*Azadirachta indica* A. Juss) seeds and relationship of azadirachtin A and oil content with rainfall, temperature and humidity. Current Science **99,** 953-956.

Ibrahim L, Butt TM, Beckett A, Clark SJ. 1999. The germination of oil-formulated conidia of the insect pathogen, *Metarhizium anisopliae*. Mycology Research. **103,** 901–907.

Ilesanmi JO, Gungula DT. 2013. Quality attributes of cowpea seeds stored with neem and Moringa seed oils. World Journal of Agricultural Science **9,** 155-160. http://dx.doi.org/10.5829/idosi.wjas.2013.9.2.1711.

Iloba BN, Ekrakene T. 2006. Comparative assessment of insecticidal effect of *Azadirachta indica, Hyptis suaveolens* and *Ocimum gratissimum*

on *Sitophilus zeamais* and *Callosobruchus maculatus*. Journal of Biological Sciences **6,** 626-630.

IRAD. 2007. Les caractéristiques des différentes zones agro-écologiques. Revue scientifique 2007 de l'IRAD.Yaoundé. 84 p.

Isman MB. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annual Review of Entomology **51,** 45-66.

Isman MB. 2008. Botanical insecticides: for richer, for poorer. Pest Management Science **64,** 8 -11.

Jadeja GC, Maheshwari RC, Naik SN. 2011. Extraction of natural insecticide azadirachtin from neem (*Azadirachta indica* A. Juss) seed kernels using pressurized hot solvent. Journal of Supercrit Fluids **56,** 253-258.
http://dx.**doi**.org/10.1016/j.supflu.2011.01.004.

Johnson S, Dureja P, Dhingra S. 2003. Photostabilizers for azadirachtin-A (a neem-based pesticide). Journal of Environmental Science and Health B **38(4),** 451-462.

Johnson S, Patra D, Dureja P. 2000. Effect of fatty acids and oils on photodegradation of Azadirachtin-A. Journal of Environmental Science and Health B **35,** 491-501.

Kapseu C, Parmentier M. 1999. Composition en acides gras de quelques huiles végétales du Cameroon. Science Alimentaire **17,** 325-331.

Kaushik N, Singh BG, Tomar UK, Naik SN, Satya V, Bisla SS, Sharma KK, Banerjee SK, Thakkar P. 2002. Regional and habitat variability in azadirachtin content of Indian neem (*Azadirachta indica* A. Jussieu). Current Science **92,** 1400-1406.

Kaushik N, Vir S. 2000. Variation of fatty acid composition of neem seed. Biochemical Society Transaction **28,** 880-882.

Kaushik N. 2002. Determination of azadirachtin and fatty acid methyl esters of *Azadirachta indica* seeds by HPLC and GLC. Analyticaland Bioanalytical Chemistry **374,** 1199-1204.

Kavallieratos NG, Athanassiou CG, Saitanis CJ, Kontodimas DC, Roussos AN, Tsoutsa MS, Anastassopoulou UA. 2007. Effect of two azadirachtin formulations against adults of *Sitophilus oryzae* and *Tribolium confusum* on different grain commodities. Journal of Food Protection **70,** 1627-1632.
http://dx.doi.org/10.1016/j.jbiotec.2012.02.017.

Kemabonta KA, Falodu BB. 2013. Bioefficacy of three plant products as post-harvest grain protectants against *Sitophilus oryzae* Linneaus (Coleoptera: Curculionidae) on stored wheat (*Triticum aestivum*). International Journal of Science and Nature **4,** 259-264.

Ketoh GK, Koumaglo HK, Glitho AI. 2005. Inhibition of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) development with essential extracted from *Cymbopogon schoenanthus* L. Spreng. (Poaceae) and the wasp *Dinarmus basalis* (Rondani) (Hymenoptera: Pteromalidae). Journal of Stored Products Research **41,** 363-371.
http://dx.doi.org/10.1016/j.jspr.2004.02.002.

Khoshnoud H, Ghiyasi M, Amimia R, Fard SS, Tajbakhsh M, Salehzadeh H, Alahyary P. 2008. The potentials of using inject properties of medicinal plants against insect pests. Pakistan Journal of Biological Sciences **11,** 1-5.

Koul O, Singh G, Singh R, Singh J, Daniewski WM, Berlozecki S. 2004. Bioefficacy and mode of action of some limonoids of salannin group from *Azadirachta indica* A. Juss and their role in a multicomponent system against lepidopteran larvae. Journal Biosciences **29(4),** 409-416.

Kumar J, Parmar BS. 1996. Physico-chemical and chemical variation in neem oils and some bioactivity

leads against *Spodoptera litura* F. Journal of Agriculture and Food Chemistry. **44,** 2137-2143.

Kumar R. 1991. La lutte contre les insectes ravageurs: la situation de l'agriculture africaine. CTA/Karthala Eds, Wageningen, Paris.

Lee HS, Shin WK, Song C, Cho KY, Ahn YJ. 2001. Insecticidal activities of ar-Turmerone identified in *Curcuma longa* Rhizome against *Nilaparvata lugens* (Homoptera: Delphacidae) and *Plutella xylostella* (Lepidoptera: Yponomeutidae). Journal of Asia Pacific Entomology **4,** 181-185.

Lienard V, Seck D, Lognay V. 1993. Biological activity of *Cassia occidentalis* L. against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). Journal of Stored Products Research **29,** 311-318.

Mbaiguinam M, Maoura N, Bianpambe A, Bono G, Aladoumbaye E. 2006. Effects of six common plant seed oils on survival, eggs lying and development of the cowpea weevil, *Callosobruchus maculatus* (F) (Coleoptera: Bruchidae). Journal of Biological Science **6,** 420-425.

Mordue AJ, Blackwell A. 1993. Azadirachtin: an update. Journal of Insect Physiology **39,** 903-924.

Muñoz-Valenzuela S, Ibarra-López AA, Rubio Silva LM, Valdez-Dávila H, Borboa Flores J. 2007. Neem tree morphology and oil content. In : Janick J, hipkey A (eds), issues in new crops and new uses. Alexendria VA: ASHS press, 126-128.

Najafian S, Agah M. 2012. Essential oil content and composition of *Lippa citriodora* as affectef by drying method before flowering stages. European Journal of Experimental Biology **2,** 1771-1777.

Niber BT. 1995. The protectant and toxicity of four plant species on stored maize against *Prostephanous truncatus* (Horn) Coleopetra: Bostrichidea. Tropical Science **35,** 371–375.

Nukenine EN, Tchiegang C, Mekouo AAT, Tofel HK, Adarkwah C, Obeng Ofori D, Adler C. 2011b. Efficacy of Calneem derived from Ghanian neem seeds and seed oils from two locations in Cameroon against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) on maize. International Journal of Tropical Insect Science **31(4),** 225-243. http://dx.doi.org/10.1017/S1742758411000348.

Nukenine EN, Adler C, Reichmuth C. 2007. Efficacy evaluation of powders from Cameroon as post harvest grain protectants against the infestation of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). Journal of Plant Diseases and Protection **114,** 30–36. http://dx.doi.org/10.1007/s10340-009-0285-6.

Nukenine EN, Chouka PF, Vabi MB, Reichmuth C, Adler C. 2013. Comparative toxicity of four local botanical powders to *Sitophilus zeamais* and influence of drying regime and particle size on insecticidal efficacy. International Journal of Biological and Chemical Science **7(3),** 1313-1325. http://dx.**doi**.org/10.4314/ijbcs.v7i3.36.

Nukenine EN, Tofel HK, Adler C. 2011a. Comparative efficacy of NeemAzal and local botanicals derived from *Azadirachta indica* and *Plectranthus glandulosus* against *Sitophilus zeamais* on maize. Journal of Pest Science **84,** 479-486. http://dx.doi.org/10.1007/s10340-011-0371-4.

Obeng-Ofori D, Amiteye S. 2005. Efficacy of mixing vegetable oils with pirimiphos-methyl against maize weevil, *Sitophilus zeamais* on stored maize. Journal of Stored Products Research **42,** 57-66. http://dx.doi.org/10.1016/j.jspr.2003.11.001.

Odeyemi OO, Gbaye OA, Akeju O. 2010. Resistance of *Callosobruchus maculatus* (Fab.) to Pirimiphos methyl in Three Zones in Nigeria. Proceeding of the 9th International Working Conference on Stored Product Protection, estoril, Portugal. 324-329.

Ogemah VK. 2003. Influence of neem products on the biology and behaviour of the larger grain borer *Prostephanus truncatus* and its predator *Teretrius nigrescens*. PhD Thesis, Humboldt University, Germany.

Pamela P. 2009. Neem the Wonder Tree: Its Pesticide and Medicinal Applications. Master thesis, University of Maryland, USA. 56 p.

Parugrug ML, Roxas AC. 2008. Insecticidal action of five plants against maize weevil, *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae) KMITL Science and Technology Journal **8,** 24 -38.

Petit JL. 2008. Le nim (ou neem), l'arbre miracle? L'insecticide se fait désirer. BIOFIL **57,** 49-51.

Radwan AO, El-Shiekh YWA. 2012. Degradation of Neem Oil 90% EC (azadirachtin) under Storage Conditions and its Insecticidal Activity against Cotton Leaf worm *S. littoralis*. Researcher **4,** 77-83.

Rembold H. 2004. Der Neembaum: Quelle für eine neue Strategie im Pflanzenschutz? Entomologie heute, **16,** 235-243.

Roy S, Gurusubramanian G, Mukhopadhyay A. 2010. Neem-based integrated approaches for the management of tea mosquito bug, Helopeltis theivora Waterhouse (Miridae: Heteroptera) in tea. Journal of Pest Science **83,** 143-148.

Rukmini C. 1987.Chemical and nutritional evaluation of neem oil. Food Chemistry **26,** 119-124.

Schmutterer H. 1990. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. Annual Review of Entomology **35,** 271-297.

Shahhoseini R, Estaji A, Hoseini N, Ghorpanpour M, Omidbaigi R. 2013. The effect of drying methods on the content and chemical composition of essential oil of *Lemon verbena* (*Lippia citriodora*). Journal of Essential Oils Bearing Plants **16,** 474. http://dx.doi.org/10.1080/0972060X.2013.813270.

Sidhu OP, Kumar V, Behl HM. 2003. Variability in neem (*Azadirachta indica*) with respect to azadirachtin content. Journal of Agriculture and Food Chemistry **51,** 910-915.

Soetaredjo FE, Budijanto GM, Prasetyo RI, and Indraswati N. 2008. Effects of pre-treatment condition on the yield and quality of neem oil obtained by mechanical pressing. ARPN-Journal of Engineering and Applied Science **3,** 45-49.

Tofel HK, Nukenine EN, Staehler M, Adler C. 2015. Insecticidal efficacy of *Azadirachta indica* powders from sun- and shade-dried seeds against *Sitophilus zeamais* and *Callosobruchus maculatus*. Journal of Entomology and Zoology Studies **3(1),** 100-108.

Tomar Uk, Singh G, Kaushik N. 2012. Screening *Azadirachta indica* tree for enhancing azadirachtin and oil contents in dry areas of Gujarat, India. Journal of Forestry Research **22,** 217-224. http://dx.doi.org/10.1007/s11676-011-0153-0.

Udo IO. 2005. Evaluation of the potential of some local spices as stored grain protectants against the maize weevil *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae). Journal of Applied Sciences and Environmental Management **9,** 165-168.

Wadehi M, El-Bakhir SS, Abdul-Safir AH. 2013. Effectiveness of neem seed oil on maize weevils *Sitophilus zeamais*. Neem report 4 p.

Jeon JH, Kim YK, Lee SG, Lee GH, Lee HS. 2011. Insecticidal activities of a *Diospyros kaki* root-isolated constituent and its derivatives against *Nilaparvata lugens* and *Laodelphax striatellus*. Journal of Asia Pacific Entomology **14,** 449-453. http://dx.doi.org/10.1016/j.aspen.2011.07.005.

Yuya AI, Tadesse A, Azerefengne F, Tefera T. 2009. Efficacy of combining Niger seed oil with malathon 5% durst formulation on maize against the maize weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae). Journal of Stored Products Research **45,** 67-70. http://dx.doi.org/10.1016/j.jspr.2008.09.003.