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Insecticidal efficacy of *Azadirachta indica* powders from sun- and shade-dried seeds against *Sitophilus zeamais* and *Callosobruchus maculatus*

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Abstract

The Azadirachtin A contents of *Azadirachta indica* powders from sun-dried kernels, shade-dried kernels, sun-dried seeds and shade-dried seeds, were determined. Cowpea or maize grains were admixed with the powders at the rates 0, 5, 10, 20, 30 and 40 g/kg for the assessment of mortality over a 6-d (*Callosobruchus maculatus*) or 14-d period (*Sitophilus zeamais*), as well as for damage and persistence bioassay. Drying regime did not affect the Azadirachtin A amounts in the seeds. All *A. indica* powders caused a significant dose-dependent mortality to *S. zeamais* and *C. maculatus*, as well as completely suppressed progeny production and grain damage. *S. zeamais* was more susceptible to the powders than *C. maculatus*. That the bioactivity of powders from sun-dried *A. indica* seeds were generally similar to those of the shade-dried ones, could speed up processing of seeds by farmers and minimize attacks by fungi which may produce aflatoxins on treated grains.

Keywords: *Azadirachta indica*, *Callosobruchus maculatus, Sitophilus zeamais,* drying regime, toxicity, persistence.

1. Introduction

Cowpea [*Vigna unguiculata* (L.) Walp] and maize (*Zea mays* L.) are among the grains that suffer from serious insect attacks during storage, especially in the tropical regions. They are respectively heavily damaged by the cowpea weevil *Callosobruchus maculatus* Fabricius (Coleoptera: Chrysomelidae) and the maize weevil *Sitophilus zeamais* Motschulsky (Coleopera: Curculionidae). *C. maculatus* is responsible for about 30 to 60% weight losses of cowpea within six months of storage [1] while 30 to 40% maize weight losses are common with *S. zeamais* [2]. To ensure food security for the whole year, farmers store more than 75% of their harvested cowpea and maize [3], and therefore, they have to fight against stored products insect pests. Different methods are used to control or to protect stored cereals and legumes against insect infestations during storage at small farmer's level as well as at the industrial level. The widely used method is the utilisation of synthetic residual pesticides which are known for their effectiveness against targeted pests [4]. But the use of these pesticides is accompanied by numerous hazards to the environment, humans and livestock [4]. The situation has necessitated the search for more acceptable method of protection of stored products. Research efforts are being encouraged on the use of botanicals insecticides because they are more biodegradable, and thus pose fewer problems to the environment [5, 6]. *Azadirachta indica* A. Juss commonly called neem, is one of the remarkable plants studied for its insecticidal efficacy against more than 500 insect species including stored products insect pests [7, 8]. The efficacy of neem seed extracts, oils or powders against insect pests is based on the ingredient Azadirachtin A [4]. In developing countries, sun-drying is employed by some people to dry plant materials [9]. However some authors have contended that sun-drying leads to photo- and thermo-degradation of the active substances in plant materials, such as Azadirachtin A in *A. indica* seeds [10]. When not properly dried, rapid contamination of *A. indica* seeds by aflatoxins occurs [11], which would in turn attain humans when consumed in treated grains, causing serious health hazards [9].

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. To promote the use of safer *A. indica* seed powder combined with good efficacy in stored product protection, the mode of drying the seeds needs to be reconsidered.

Therefore, the present study was designed to compare the Azadirachtin A contents of *A. indica* powders obtained from seeds that were subjected to four drying regimes (sun-dried seeds, sun-dried kernels, shade-dried seeds and shade dried kernels). The adult mortality and progeny inhibition of *S. zeamais* on maize and *C. maculatus* on cowpea that were treated with the four powders were determined. In addition, the insects' damage reductions on the treated grains were also assessed.

2. Materials and methods

2.1 Insect

S. 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 of Julius Kühn-Institut (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. This work was carried out from October 2012 to April 2013 in the Laboratory of Stored Products Protection at the JKI Berlin, Germany.

2.2 Collection and processing of *Azadirachta indica* seeds

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 [12]. 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 relative humidity 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).

In Berlin, the dried kernels of *A. indica* were ground into powder using a Bosch Universal grinder (MUM 6012, Remscheid, Germany) until the particles passed through a 1mm mesh sieve. The obtained powders were introduced into an opaque glass and stored in a deep-freezer at -18 °C until needed for bioassay.

2.3 Determination of Azadirachtin A content in *A. indica* seeds

Extraction and cleanup of the *A. indica* seed powders from the different drying regimes were carried out using QuEChERS [13]. 100 µl of powder or 2 g of powder were 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 mg/µl and subsequently kept in the dark at 4 °C until analyzed by LC/MS/MS. According to the drying method, each treatment was replicated three times and for each tube two replications were 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.

2.4 Adult toxicity test and F_1 progeny production

The weight of 0.25, 0.5, 1, 1.5 and 2 g of *A. indica* powders from sun-dried seeds, sun-dried kernels, shade-dried seeds and shade-dried kernels were separately introduced into 50 g of maize or cowpea in 250 ml glass jars to give the contents of 5, 10, 20, 30 and 40 g/kg of maize or cowpea. Controls consisted of grains without *A. indica* seed powder. Each jar was shaken with a bidimensional mixer (Gerhardt, Dreieich, Germany) for approximately 4 min to ensure uniform distribution of the powders to the entire grain mass. Groups of 20 *S. zeamais* or *C. maculatus* were separately added to glass jars containing the treated maize or cowpea, respectively. Control glass 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 [14].

2.5 Grain damage
Similar dosages of each type of *A. indica* powder according to the drying regime, as for the toxicity bioassay described above, were considered for 100 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 or cowpea and the number of damaged grains (grains with characteristic holes) and undamaged grains were counted and weighed. Percent weight loss (%WL) was computed using FAO [15] method as follows:

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

Where, U is the weight of undamaged grains, U*a* is the weight of the damaged grains, N*a* is the number of damaged grains; N*e* is the number of undamaged grain.
The Percentage grain damage (%D) was therefore, calculated using the formula:

$$\%D = (B/A) \times 100$$

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

2.6 Persistence of *A. indica* powder on grains
Maize and cowpea grains were treated with *A. indica* powder from the sun-dried kernels in the same order as described previously for toxicity bioassays. Twenty adult beetles were exposed to the treated grains (maize or cowpea) which had been stored for 0, 30, 60 and 180 days. Mortality counts were carried out 3 and 5 days after exposure for *C. maculatus* and *S. zeamais,* respectively. All treatments were replicated four times.

2.7 Data analysis
The mortality counts were corrected with Abbott's formula [16]. 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 ($\log_{10}$ x+ 1), 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.

3. Results
3.1 Azadirachtin A contents of *A. indica* powder from seeds exposed to different drying regimes
The Azadirachtin A contents from *A. indica* seeds that were subjected to the four drying regimes were similar ($F_{3, 8} = 2.54$, $P > 0.05$) (Table 1).

Table 1: Azadirachtin A content of *Azadirachta indica* seed powders that were subjected to different drying regimes

Drying regime	Azadirachtin (g/kg)
Shade-dried kernels	1.20 ± 0.02
Sun-dried kernels	1.19 ± 0.07
Shade-dried seeds	1.54 ± 0.26
Sun-dried seeds	1.05 ± 0.03
$F_{(3, 8)}$ ‡	2.54^{ns}

‡ ns = $P > 0.05$

3.2 Toxicity tests
The results of the toxicity test showed that compared to the control, all the *A. indica* seed powders generally caused significant dose-dependent mortality to adult *C. maculatus* and *S. zeamais* (Tables 2 and 3). *S. zeamais* was more susceptible to the seed powders than *C. maculatus*, irrespective of the drying regime. Maximum mortality caused by the seed powders to *C. maculatus* was 34.28% within 6 days of exposure. Greater than 90% mortality of *S. zeamais* was achieved with the highest dosage (40 g/kg) for all the powers within 7 days of exposure. Complete mortality (100%) of the weevil was generally recorded within 14 days of exposure at the highest tested dosage. For *C. maculatus*, there were no clear trends in mortality of the powders linked to sun- or shade-drying. However, the insects tended to be more susceptible to the powders from the dried kernels as compared to the dried seeds. Sun-drying appeared to enhance the potency of the *A. indica* seeds and kernels towards *S. zeamais* at higher dosage levels.
The 3-day LC_{50} values given by extrapolation for *C. maculatus* were 83.27 g/kg (sun-dried seeds), 86.27 g/kg (shade-dried kernels), 96.07 g/kg (shade-dried seeds) and 105.94 g/kg (sun-dried kernels), and the 7-day LC_{50} values for *S. zeamais* were 8.14 g/kg for the sun-dried seeds, 10.75 g/kg for the shade-dried kernels, 11.57 g/kg for the sun-dried kernels and 14.41 g/kg for the shade-dried seeds.

Table 2: Corrected cumulative mortality of adult *Callosobruchus maculatus* exposed in grains treated with *Azadirachta indica* seed powders obtained from seeds that were subjected to different drying regimes

Exposure period (days)	Dose (g/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	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
	5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
	10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
	20	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
	30	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
	40	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
	$F_{(5, 18)}$ ‡	–	–	–	–	
3	0	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	–

	10	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	–
	20	5.00 ± 2.04^{bc}	2.50 ± 1.44^{bc}	2.50 ± 1.44^{bc}	0.00 ± 0.00^{b}	2.00 ns
	30	15.00 ± 3.54^{aA}	5.00 ± 2.04^{abB}	5.00 ± 0.00^{bB}	3.75 ± 1.25^{aB}	6.03*
	40	15.00 ± 00^{aA}	11.25 ± 1.25^{aAB}	12.50 ± 1.44^{aAB}	7.50 ± 1.44^{aB}	6.82*
	$F_{(5, 18)}$ ‡	32.52**	12.50***	28.42***	21.51***	
6	0	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	–
	5	1.32 ± 1.32^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	1.00 ns
	10	7.57 ± 1.41^{bc}	6.32 ± 1.23^{c}	3.75 ± 2.39^{cd}	6.32 ± 1.23^{b}	0.96 ns
	20	20.26 ± 2.06^{bA}	15.20 ± 2.05^{bAB}	10.00 ± 2.89^{bcB}	13.88 ± 2.33^{abAB}	3.24*
	30	31.65 ± 3.12^{aA}	22.83 ± 1.66^{bA}	12.50 ± 1.44^{abB}	13.82 ± 3.70^{abB}	11.24***
	40	30.46 ± 2.43^{a}	34.28 ± 4.07^{a}	23.75 ± 2.39^{a}	22.76 ± 3.17^{a}	3.17 ns
	$F_{(5, 18)}$ ‡	62.13***	109.59***	24.85***	40.49***	

† 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

Table 3: Corrected cumulative mortality of adult *Sitophilus zeamais* exposed in grains treated with *Azadirachta indica* seed powders obtained from seeds that were subjected to different drying regimes

Exposure period (days)	**Dose (g/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^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	
	5	1.25 ± 1.25^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	1 ns
	10	3.75 ± 2.39^{bc}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	2.45 ns
	20	5.00 ± 2.04^{abcA}	1.25 ± 1.25^{bAB}	0.00 ± 0.0^{bB}	0.00 ± 0.00^{bB}	3.91 *
	30	8.75 ± 1.25^{abA}	2.50 ± 1.44^{bAB}	1.25 ± 1.25^{bB}	5.00 ± 2.04^{aAB}	4.67 *
	40	15.00 ± 2.04^{aA}	8.75 ± 2.39^{aAB}	6.25 ± 1.25^{aB}	7.50 ± 1.44^{aAB}	4.46 *
$F_{(5, 18)}$ ‡		8.30***	8.67***	15.93 ***	16.76*	
3	0	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	
	5	5.00 ± 3.54^{de}	5.00 ± 2.04^{cd}	1.25 ± 1.25^{c}	7.50 ± 4.33^{bc}	0.72 ns
	10	13.75 ± 2.39^{bc}	16.25 ± 2.39^{c}	15.00 ± 1.09^{b}	17.50 ± 1.44^{ab}	0.59ns
	20	22.50 ± 4.79^{bc}	42.50 ± 9.24^{b}	26.25 ± 2.39^{b}	23.75 ± 4.73^{ab}	2.53 ns
	30	36.25 ± 5.15^{abBC}	63.75 ± 3.15^{abA}	53.75 ± 2.39^{aAB}	30.00 ± 5.40^{aC}	13.57***
	40	47.50 ± 4.33^{aB}	70.00 ± 2.04^{aA}	67.50 ± 3.23^{aA}	38.75 ± 5.54^{aB}	14.53***
$F_{(5, 18)}$ ‡		26.21***	54.65***	141.06***	10.93 ***	
7	0	0.00 ± 0.00^{e}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	
	5	22.50 ± 7.46^{d}	15.20 ± 5.40^{b}	12.50 ± 1.44^{c}	36.25 ± 6.88^{d}	2.69 ns
	10	33.75 ± 6.25^{cd}	26.91 ± 2.40^{b}	30.00 ± 3.54^{bc}	52.50 ± 12.67^{cd}	2.42 ns
	20	66.25 ± 10.08^{bcAB}	84.74 ± 3.41^{aA}	50.00 ± 5.40^{bB}	78.75 ± 4.73^{bcA}	5.73*
	30	85.00 ± 2.04^{ab}	95.25 ± 4.75^{a}	86.25 ± 6.25^{a}	88.75 ± 4.73^{ab}	0.95 ns
	40	92.50 ± 4.79^{a}	97.50 ± 2.50^{a}	98.75 ± 1.25^{a}	97.50 ± 1.44^{a}	0.94 ns
$F_{(5, 18)}$ ‡		32.54***	64.89***	82.63***	44.61*	
14	0	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{c}	
	5	28.95 ± 7.62^{cAB}	28.03 ± 5.00^{cAB}	21.51 ± 1.19^{dB}	50.72 ± 5.67^{bA}	5.33*
	10	52.90 ± 7.39^{b}	53.82 ± 7.51^{b}	47.90 ± 9.45^{c}	68.55 ± 8.13^{b}	1.19 ns
	20	84.74 ± 6.53^{aA}	98.75 ± 1.25^{aA}	67.04 ± 3.38^{bB}	98.75 ± 1.25^{aA}	15.85 ***
	30	97.37 ± 2.63^{a}	100 ± 0.00^{a}	98.69 ± 1.32^{a}	100.00 ± 0.00^{a}	0.73 ns
	40	98.69 ± 1.32^{a}	100 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	1.00 ns
$F_{(5, 18)}$ ‡		54.28***	209.69***	151.80***	160.04***	

† 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 $P > 0.05$, * $P < 0.05$; *** $P<0.001$

3.3 F_1 Progeny production

All the dosages of the *A. indica* seed powders from the four drying regimes completely suppressed the F_1 progeny emergence in *C. maculatus* on treated cowpea (Table 4). Somewhat similar results of progeny suppression by the powders were obtained for *S. zeamais* (Table 5).

Table 4: Progeny production of *Callosobruchus maculatus* in grains treated with *Azadirachta indica* seed powders obtained from seeds that were subjected to different drying regimes

Dose (g/kg)	Drying regime				$F_{(3, 12)}$ ‡
	Shade-dried kernels	Sun-dried kernels	Shade-dried seeds	Sun-dried seeds	
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	0.95 ns
5	0.00 ± 0.00^b	0.00 ± 0.00^b	0.00 ± 0.00^b	0.00 ± 0.00^b	–
10	0.00 ± 0.00^b	0.00 ± 0.00^b	0.00 ± 0.00^b	0.00 ± 0.00^b	–
20	0.00 ± 0.00^b	0.00 ± 0.00^b	0.00 ± 0.00^b	0.00 ± 0.00^b	–
30	0.00 ± 0.00^b	0.00 ± 0.00^b	0.00 ± 0.00^b	0.00 ± 0.00^b	–
40	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	–
5	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	–
10	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	–
20	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	–
30	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	–
40	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

Table 5: Progeny production of *Sitophilus zeamais* in grains treated with *Azadirachta indica* seed powders obtained from seeds that were subjected to different drying regimes

Dose (g/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	39.50 ± 2.66^a	46.50 ± 8.87^a	42.25 ± 0.95^a	42.50 ± 1.94^a	0.50 ns
5	0.75 ± 0.75^b	0.00 ± 0.00^b	0.75 ± 0.48^b	0.50 ± 0.50^b	0.48 ns
10	0.00 ± 0.00^b	0.00 ± 0.00^b	0.00 ± 0.00^b	0.00 ± 0.00^b	–
20	0.00 ± 0.00^b	0.00 ± 0.00^b	0.00 ± 0.00^b	0.00 ± 0.00^b	–
30	0.00 ± 0.00^b	0.00 ± 0.00^b	0.00 ± 0.00^b	0.00 ± 0.00^b	–
40	0.00 ± 0.00^b	0.00 ± 0.00^b	0.00 ± 0.00^b	0.00 ± 0.00^b	–
$F_{(5, 18)}$ ‡	238.85***	891.80***	655.65***	488.88***	
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	
5	98.37 ± 1.63^a	100 ± 0.00^a	98.17 ± 1.17^a	98.81 ± 1.19^a	0.60 ns
10	100 ± 0.00^a	100 ± 0.00^a	100 ± 0.00^a	100.00 ± 0.00^a	–
20	100 ± 0.00^a	100 ± 0.00^a	100 ± 0.00^a	100.00 ± 0.00^a	–
30	100 ± 0.00^a	100 ± 0.00^a	100 ± 0.00^a	100.00 ± 0.00^a	–
40	100 ± 0.00^a	100 ± 0.00^a	100 ± 0.00^a	100.00 ± 0.00^a	–
$F_{(5, 18)}$ ‡	583.43***	–***	759.32***	805.66***	

† 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

3.4 Grain damage

The damage and weight loss of cowpea and maize grains that were treated with the four drying regimes of *A. indica* seed powder, infested and stored for 10 weeks were statistically different from those of the control (P = 0.0001) (Tables 6 and 7). Generally, the damage caused by *C. maculatus* to cowpea seeds, as well as the resulting weight losses were lower across the four drying regimes compared to that caused on maize by *S. zeamais*. Apart from the cowpea grains treated with the lowest dosage (5 g/kg) of *A. indica* seed powders, which suffered little damage and weight loss caused by *C. maculatus*, the treated grains recorded no grain damage and weight loss (Table 6). Maize grains recorded damage (0.25 - 10.50%) and weight losses (0.02 - 2.78%) when treated with the different dosages of the *A. indica* seed powders depending on the drying regime. Also, maize treated with the powders from the *A. indica* from the different drying regimes suffered insignificant or no damage and weight loss when the dose level was ≥ 30 g/kg (Table 7). Nonetheless, the sun-drying of seeds led to a higher damage and weight loss of cowpea and maize at the lowest tested dosage of 5 g/kg.

Table 6: Grain damage and weight loss of cowpea caused by *Callosobruchus maculatus* in grains treated with *Azadirachta indica* seed powder obtained from seeds that were subjected to different drying regimes and then stored for 10 weeks storage

Doses (g/kg)	Drying regime				
	Shade-dried kernels	Sun-dried kernels	Shade-dried seeds	Sun-dried seeds	$F_{(3, 12)}$ ‡
Mean (± SE) grain damage (%) †					
0	98.25 ± 0.25^{a}	97.75 ± 0.63^{a}	98.50 ± 0.29^{a}	98.00 ± 0.41^{a}	0.50 ns
5	0.50 ± 0.50^{bB}	0.00 ± 0.00^{bB}	5.75 ± 2.25^{bAB}	9.00 ± 2.48^{bA}	6.52**
10	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	–
20	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	–
30	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	–
40	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	–
$F_{(5, 18)}$ ‡	1501.19***	1858***	858.80***	301.51***	
Mean (± SE) weight loss (%) †					
0	48.05 ± 1.54^{aAB}	38.42 ± 4.61^{aB}	51.52 ± 1.57^{aAB}	52.57 ± 3.66^{aA}	4.20*
5	0.05 ± 0.05^{bB}	0.00 ± 0.00^{bB}	1.24 ± 0.42^{bA}	0.85 ± 0.28^{bAB}	5.79 **
10	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	–
20	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	–
30	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	–
40	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	–
$F_{(5, 18)}$ ‡	1580.47***	201.31***	1053.75***	353.83***	

† 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

Table 7: Grain damage and weight loss of maize caused by *Sitophilus zeamais* in grains treated with *Azadirachta indica* seed powders obtained from seeds that were subjected to different drying regimes and then stored for 10 weeks storage

Doses (g/kg)	Drying regime †				
	Shade-dried kernels	Sun-dried kernels	Shade-dried seeds	Sun-dried seeds	F $F_{(3, 12)}$ ‡
Mean (± SE) grain damage (%) †					
0	49.75 ± 1.03^{a}	52.00 ± 3.03^{a}	45.25 ± 3.28^{a}	46.00 ± 2.20^{a}	1.55 ns
5	2.75 ± 1.03^{bB}	3.25 ± 1.60^{bcB}	3.25 ± 0.75^{bB}	10.50 ± 1.26^{bA}	9.57**
10	2.50 ± 0.29^{c}	4.25 ± 0.95^{b}	2.25 ± 0.48^{b}	4.75 ± 1.70^{bc}	1.52 ns
20	0.75 ± 0.48^{c}	2.00 ± 0.71^{bcd}	1.50 ± 1.50^{b}	2.00 ± 0.91^{cd}	0.37 ns
30	0.50 ± 0.29^{c}	0.75 ± 0.48^{cd}	1.75 ± 0.85^{b}	0.00 ± 0.00^{d}	2.07 ns
40	0.75 ± 0.48^{c}	0.00 ± 0.00^{d}	0.25 ± 0.25^{b}	0.00 ± 0.00^{d}	1.71 ns
$F_{(5, 18)}$ ‡	102.03***	83.87***	51.82***	103.66***	
Mean (± SE) weight loss (%) †					
0	12.40 ± 0.30^{a}	11.41 ± 1.41^{a}	12.33 ± 1.30^{a}	8.93 ± 2.95^{a}	0.84 ns
5	0.53 ± 0.18^{bB}	0.60 ± 0.17^{bB}	0.72 ± 0.24^{bB}	2.78 ± 0.23^{bA}	27.70***
10	0.32 ± 0.29^{b}	0.95 ± 0.28^{b}	0.38 ± 0.07^{b}	1.56 ± 0.62^{bc}	2.82 ns
20	0.23 ± 0.16^{b}	0.32 ± 0.18^{bc}	0.08 ± 0.08^{b}	0.35 ± 0.17^{bc}	0.62 ns
30	0.21 ± 0.12^{b}	0.02 ± 0.02^{d}	0.64 ± 0.32^{b}	0.00 ± 0.00^{c}	2.92 ns
40	0.00 ± 0.00^{b}	0.00 ± 0.00^{d}	0.08 ± 0.08^{b}	0.00 ± 0.00^{c}	1.58 ns
$F_{(5, 18)}$ ‡	66.25***	71.20***	59.14***	11.44***	

† 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 $P > 0.05$, * $P < 0.05$, *** $P < 0.001$

3.5 Persistence on grains

The persistence of powder obtained from sun-dried kernels on treated cowpea or maize showed that its potency decreased significantly with storage time and dosage (Table 8). The mortality caused to *C. maculatus* ≥ 30 days after treatment differed from those registered at 0 day ($P < 0.05$) when the dose level was ≥ 20 g/kg. Except the dosages 30 and 40 g/kg, sun-dried *A. indica* seed powder on maize grains that has been stored for 0, 30 and 60 days caused similar adult mortality to *S. zeamais*, but the mortality significantly declined by 180 days of storage. At the highest dose (40 g/kg), mortality of *C. maculatus* on cowpea decreased from 17.50% (0 day) to 0.00% (60 days). Maximum mortality of 26.25% (40 g/kg) for *S. zeamais* was recorded on treated grains that have been stored for 180 days.

Table 8: Corrected cumulative mortality of *Callosobruchus maculatus* and *Sitophilus zeamais* exposed in grains treated with neem seed powder after different periods of storage

Insects and doses (g/kg)		Infestation period (days) / % Mortality (mean ± SE) †				
		0	30	60	180	$F_{(3, 12)}$‡
Callosobruchus maculatus						
0		0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	
5		0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	–
10		3.75 ± 3.75^{bc}	0.00 ± 0.00^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1ns
20		5.00 ± 3.02^{abcA}	0.00 ± 0.00^{bB}	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aB}	6.00*
30		12.50 ± 3.23^{abA}	1.25 ± 1.25^{abB}	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aB}	12.30***
40		17.50 ± 3.23^{aA}	3.75 ± 1.25^{aB}	0.00 ± 0.00^{aC}	0.00 ± 0.00^{aC}	32.11***
$F_{(5, 18)}$‡		10.20***	4.40**	ns	ns	
Sitophilus zeamais						
0		0.00 ± 0.00^{e}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	
5		10.00 ± 2.04^{dA}	7.50 ± 2.50^{bAB}	10.00 ± 4.08^{bcA}	0.00 ± 0.00^{cB}	4.68*
10		25.00 ± 4.56^{cAB}	36.25 ± 12.48^{aA}	31.25 ± 10.87^{abAB}	5.00 ± 3.54^{bcB}	3.88*
20		50.00 ± 3.54^{bA}	45.00 ± 3.54^{aA}	47.50 ± 5.95^{aA}	5.00 ± 2.04^{bcB}	32.13***
30		73.75 ± 2.39^{aA}	52.50 ± 3.23^{aAB}	41.25 ± 6.88^{aB}	18.75 ± 5.15^{abC}	21.61***
40		82.50 ± 4.79^{aA}	61.25 ± 4.27^{aAB}	46.25 ± 8.98^{aBC}	26.25 ± 10.68^{aC}	9.41**
$F_{(5, 18)}$‡		106.34***	25.18***	13.40***	8.39**	

† 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 is not possible due to equal variance

4. Discussion

The Azadirachtin content of *A. indica* seeds vary across geographical locations [17, 18, 19]. The range 1.05 - 1.54 g/kg of Azadirachtin A contents of *A. indica* seeds in the present study is within the range reported in the literature, although it is more on the lower side. Gruber [26], Boursier *et al.* [19] and Faye (2010) [18], respectively analyzed the *A. indica* seeds from Nicaragua, Mali and Senegal and correspondingly recorded 4.0 g/kg, 3.5 g/kg and 2.0 g/kg Azadirachtin A. Sidhu *et al.* [20] studied the variation of Azadirachtin A in *A. indica* seeds of 43 provenances in India, and recorded a range from 0.55 to 3.03 g/kg of Azadirachtin A with only those from four provenances reached the rate 2.00 g/kg. Thus, the result with the Cameroonian *A. indica* seeds in the present work is in accordance with that from most of the Indian regions. The differences in the Azadirachtin contents of *A. indica* seeds may be explained by the variation of the geographical locations [21]. Soils and climate may influence the Azadirachtin A amounts in the seeds [20].

It is widely reported that sun-drying of plant materials has a negative effect on their chemical composition and thus reduces their efficacy when used as insecticides or medication [22, 23, 24]. Our results show that the contents of Azadirachtin A were similar among *A. indica* powders obtained from sun- and shade-dried seeds and kernels, indicating that sun-drying did not degrade nor deteriorate the limonoid.

The higher mortality caused by the seed powders to *S. zeamais* compared with *C. maculatus* indicate that the former was more susceptible to the powders than the latter. This was probably due to the different feeding behavioral of the insects [7]. As a bruchid, *C. maculatus* did not feed on the cowpea seeds while adult *S. zeamais* fed on maize. Therefore, a greater quantity of the powders penetrated *S. zeamais* through ingestion and probably through the cuticles, but *C. maculatus* could get a minute quantity of the powder through the cuticle. The intake of powder during feeding might act as stomach poison which led to the higher death rate of the adult insects in the case of *S. zeamais* [25]. The ingested Azadirachtin A in *A. indica* powder may inhibit cell proliferation and RNA synthesis, which results to the direct cell death, and thus the death of the weevils [26].

The mortality in both insects increased with time post-exposure and dose levels, although with a much lower rate for *C. maculatus*. This mortality may be related partially to the physical action of the neem powders particularly for *C. maculatus* since the adult do not feed on seeds. Neem seed powder is oily, and when in contact with insect may obstruct some spiracles of the insect, and then with time, led to asphyxiation and death ensues [27]. This is thought to be one of the mechanisms in which neem seed powder caused the death of both insects especially for *C. maculatus* in the present study. These results corroborate the findings of Bamaiyi *et al.* [28] who recorded lower mortality in *C. maculatus* (less than 30% at 30 g/kg) with *Khaya senegalensis* seed powder as compared to the cowpea weevils in the current study. However, Iloba and Ekrakene [7], found that adult *C. maculatus* was more susceptible to *A. indica* leaf powder than *S. zeamais*. Adult bruchids may live longer without food than adult *S. zeamais*. Thus, the higher rate of increase in adult *S. zeamais* mortality with time could also be correlated with antifeedant activity. Through a reduction or absence of food intake due to the bitter taste and the bad odour of neem powder caused by its limonoid constituents, culminate in death of the species [4, 29].

No clear trends in mortality of *S. zeamais* or *C. maculatus* linked to the sun- or shade-drying of the *A. indica* seeds were observed, which is in line with the similar Azadirachtin A contents among the different seed powders according to drying regime. Sun-stored and room-stored *A. indica* seeds from Sudan had similar efficacy on *Tribolium castaneum* [30]. The present result confirms El Shafie and Almahy [30] studies who stated that sun or shade-drying of *A. indica* seeds does not affect the effectiveness of the products on cowpea and maize weevils.

One of the fundamental characteristics of an effective grain protectant is its ability to reduce progeny production of insects in the treated grains [31]. The comparative effectiveness of powder obtained from the four drying regimes of *A. indica* seeds to inhibit F_1 progeny showed botanicals were highly effective against *C. maculatus* and *S. zeamais*, as very little or no emergence was observed even with the lowest dose level of 5 g/kg, irrespective of the drying regime of the seeds, thus lending credence to the fact that sun- drying does not deteriorate nor degrade compounds in *A. indica* seeds. This result is in accord with those of other workers. Lale and Abdulrahman [32] reported that neem seed powder or oil reduced progeny production respectively of *C. maculatus* and *S. zeamais*. Powders of *Calotropis procera* and of *Senna occidentalis* L. reduced the F_1 progeny production of *Caryedon serratus* on groundnut by 99% [33]. Udo [34] stated that, there is a relationship between F_1 progeny emergence and adult mortality. Since no mortality was observed in cowpea weevil, at the dose of 5 g/kg and no adult emerged, this statement could not be valid for *A. indica* seed powder applied on cowpea in the present study. Suppression of progeny may have been achieved through a combination of oviposition deterrence, high mortality of eggs, larvae and nymphs [32]. As the *A. indica* seed powder is oily, it has the ability to penetrate the chorion of Bruchid eggs via the micropyle and oil might occlude egg funnel which blocks respiration of the egg leading to the asphyxiation of developing insect [35]. Some compounds like azadirachtin, nimbin and salanin have the possibility of infiltrating the grains. Thus, when larvae and nymphs come in contact with toxic compound they will die during their ecdysis since the metamorphosis period is strongly affected by such molecules and causes many morphogenetics defects as well as mortality suggesting that penetration of these limonoids could affect the larval and nymphal viability of *S. zeamais* and *C. maculatus* [36].

Cowpea and maize suffer a great damage 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 were damaged. Negligible or no damage nor weight loss were recorded in maize and cowpea seeds that were treated with the *A. indica* seed powders (> 5 g/kg), respectively, regardless of the drying regime. Again, sun-drying had no negative effect on the potency of *A. indica* seed powders. Similar pattern of low seed damage was noticed with rubber seed oil and palm seed oil when applied against *C. maculatus* [37]. The reduction of damage in our study is the consequence of the antifeedant, oviposition deterrence, ovicidal, larvicidal and reproduction inhibitory properties of *A. indica* [4, 29, 32]

The decline of the residual toxicity of the *A. indica* seed powder was sharper on cowpea than on maize, with the mortality of *C. maculatus* reducing from 17.50%, without storage to 0.00 % mortality after 60 days of storage at the dosage of 40 g/kg and that of *S. zeamais* from 85.50% to 26.25%. This difference could be due firstly to the seed coat of the treated grains. Cowpea seed coat from this study is thinner and more permeable to oils, thus allowing the oils in *A. indica* powder to enter into the seed, while maize seed coat is less permeable to oils. Through this mechanism of permeability, the physical contact between insect and active ingredients and oil is reduced and limited mortality of *C. maculatus* by anoxia compared to *S. zeamais*. This is compounded by the fact that the large particle size (1 mm) of *A. indica* seed powder would not completely cover the insect's body for suffocation to occur. *S. zeamais* adults feed on grains, and took in compounds of *A. indica* powder, which could cause the death of the adult insect, lending a longer residual toxicity of the powder to the insect. Similar results were registered with *Jatropha* seed oil on cowpea within the same period of storage by Boateng and Kusi [38]. The decrease in the residual toxicity of the *A. indica* powder could also be linked to the degradation of its main compound, Azadirachtin A. Boursier *et al.* [19] mentioned that if Azadirachtin A is stored at 25 °C, its content can stay stable at least between 7 and 14 days. So, as the efficacy of the powder persisted up to more than two months, it means that Azadirachtin A could stay stable for more than one month or neem powder may contained some molecules which caused maize weevil mortality after degradation of its main constituents. Therefore, it is important to analyze the rate of neem limonoid compounds on cowpea and maize treated with *A. indica* products over time, so as to have an idea on its degradation rate, since it is known that neem products had no side effect on human when it is taken at a dose of 177 mg/kg daily [39].

Sun-drying of *A. indica* seeds had no significant effect on the bio-efficacy of the powder against *C. maculatus* and *S. zeamais* on cowpea and maize grains, respectively. All the powders obtained from *A. indica* seeds following the different drying regimes greatly protected the grains against the infestation of their respective pest insect. Since *A. indica* grows in countries where high temperatures of up to 40 °C are common, sun-drying of *A. indica* seed could be a suitable method for subsistence farmers to obtain safer and cheaper botanicals for the protection of cowpea and maize grains against insect attacks during storage.

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6. Reference

1. Ketoh GK, Koumaglo HK, Glitho AI. 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 2005; 41: 363-371.
2. Yuya AI, Tadesse A, Azerefengne F, Tefera T. Efficacy of combining Niger seed oil with malathion 5% durst formulation on maize against the maize weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae). Journal of Stored Products Research 2009; 45:67-70.
3. Kumar R. La lutte contre les insectes ravageurs: la situation de l'agriculture africaine. CTA/Karthala, Wageningen, Paris, 1991.
4. Isman MB. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annual Review of Entomology 2006; 51:45-66.
5. Boeke SJ, Baumgart IR, Van Loon JJA, Van Huis A, Dicke M, Kossou DK. Toxicity and repellence of African plants traditionally used for the protection of stored cowpea against Callosobruchus maculatus. Journal of Stored Products Research 2004; 40:423–438.
6. Boulogne I, Petit P, Ozier-Lafontaine, Desfontaines L, Loranger-Merciris G. Insecticidal and antifungal chemicals produced by plants: a review. Environ Chem Lett 2012; 10:325- 347.
7. Iloba BN, Ekrakene T. Comparative assessment of insecticidal effect of *Azadirachta indica, Hyptis suaveolens* and *Ocimum gratissimum* on *Sitophilus zeamais* and *Callosobruchus maculatus*. Journal of Biological Science 2006; 6:626-630.

8. Debashri M, Tamal M. A review on efficacy of *Azadirachta indica* A. Juss based biopesticides: An Indian perspective. Research Journal of Recent Sciences 2012; 1:94-99.
9. Nukenine EN, Tofel HK, Adler C. Comparative efficacy of Neem Azal and local botanicals derived from *Azadirachta indica* and *Plectranthus glandulosus* against *Sitophilus zeamais* on maize. Journal of Pest Science 2011; 84:479-486.
10. Koul O, Wahab S. Neem biotechnology- A synthesis. In: Koul O, Wahab S (eds) Neem: Today and in the New Millenium, Kluwey Academic, Dordrecht, 2004; 243-259.
11. Kaushik N, Singh BG, Tomar UK, Naik SN, Satya V, Bisla SS, *et al.* Regional and habitat variability in azadirachtin content of Indian neem (*Azadirachta indica* A. Jussieu). Current Science 2002; 92:1400-1406.
12. IRAD. Les caractéristiques des différentes zones agro-écologiques. Revue scientifique 2007 de l'IRAD, Yaoundé, 2007.
13. Anastassiades M, Lehotay SJ, Štajnbaher D, Schenck FJ. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. J AOAC Int 2003; 86:412-431.
14. Nukenine EN, Adler C, Reichmuth C. Efficacy evaluation of powders from Cameroon as postharvest grain protectants against the infestation of S*itophilus zeamais* Motschulsky (Coleoptera: Curculionidae). Journal of Plant Diseases and Protection 2007; 114:30-36.
15. FAO. Prevention of post-harvest food losses. Training series N°10, Food and Agricultural Organization of the United Nations, Rome, 1985.
16. Abbott WS. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 1925; 18:265-267.
17. Gruber AK. Wachstum, Fruchertrag und Azadirachtingehalt der Samen von *Azadirachta indica* A. Juss auf verschiedenen Standorten in Nicaragua. PhD Thesis, Technische Universitaet Berlin. 1991.
18. Faye M. 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. 2010.
19. Boursier CM, Bosco D, Coulibaly A, Negre M. Are traditional neem extract preparations as efficient as a commercial formulation of azadirachtin A? Crop Ptotection 2011; 30:318-322.
20. Sidhu OP, Kumar V, Behl HM. Variability in neem (*Azadirachta indica*) with respect to azadirachtin content. Journal of Agricultural and Food Chemistry 2003; 51:910-915.
21. Ermel KE, Pahlich E, Schmutterer H. Azadirachtin content of neem kernels from different geographical locations, and its dependence on temperature, relative humidity and light. Proc. 3rd Int Neem Conf Nairobi, Kenya 1986; 171-184.
22. Caboni PL, Sarais G, Angioni A, Lai F, Dedola F, Cabras P. Fate of azadirachtin A and related azadirachtoids on tomatoes after greenhouse treatment. Journal of Environmental Science and Health 2009; 44:598-605.
23. Najafian S, Agah M. Essential oil content and composition of *Lippia citriodora* as affected by drying method before flowering stages. European Journal of Experimental Biology 2012; 2:1771-1777.
24. Shahhoseini R, Estaji A, Hoseini N, Ghorpanpour M, Omidbaigi R. The effect of drying methods on the content and chemical composition of essential oil of Lemon verbena (*Lippia citriodora*). Journal of Essential Oil Bearing Plants 2013; 16:474-481.
25. Mulungu LS, Lupenza G, Reuben SOWM, Misangu RN. Evaluation of botanical products as stored grain protectant against maize weevil, *Sitophilus zeamais* (L.) on maize. Journal of Entomology 2007; 2:258-262.
26. Fritzsch U, Cleffmann G. The insecticide azadirachtin reduces predominantly cellular RNA in *Tetrahymena*, Naturwissenschaften 1984; 74:191.
27. Reuben SOMW, Masunga M, Makundi R, Misangu RN, Kilonzo B, Mwatawala M *et al.* Control of cowpea weevil (*Callosobruchus maculatus* F.) in stored cowpea (*Vigna unguiculata* L.) grains using botanicals. Asian Journal of Plant Sciences 2006; 5:91-97.
28. Bamaiyi LJ, Ndams IS, Toro WA, Odekina S. Effect of mahogany *Khaya senegalensis* seed oil in the control of *Callosobruchus maculatus* on stored cowpea. Plant Protection Science 2006; 42:130-134.
29. Manikanta P, Dokuparthi SSK. A review on role of *Azadirachta indica* A. Juss as a biopesticide. International Journal of Universal Pharmacy and Biosciences 2014; 3:2319-8141.
30. El-Shafie HAF, Almahy AAM. Effect of storage conditions and duration on the potency of Neem (*Azadirachta indica* A. Juss) seeds as home-made insecticide. Agriculture and Biology Journal of North America 2012; 3:385-390.
31. Khoshnoud H, Ghiyasi M, Amimia R, Fard SS, Tajbakhsh M, Salehzadeh H. The potential of using insecticidal properties of medicinal plants against insects pests. Pakistan Journal of Biological Sciences 2008; 11:1-5.
32. Lale NES, Abdulrahman HT. Evaluation of neem (*Azadirachta indica* A. Juss) seed oil obtained by different methods and neem powder for the management of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in stored cowpea. Journal of Stored Products Research 1999; 35:135-143.
33. Thiaw C, Gueye S, Ndiaye G, Samb A, Sembène M. Ovicid and adulticid effects of powders and extracts of *Calotropis procera* AIT. and of *Senna occidentalis* L. on *Caryedon serratus* (OL.) destroyer of groundnut stocks. Journal of Sciences 2007; 7:1-15.
34. Udo IO. 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 2005; 9:165-168.
35. Copping GL, Menn JJ. Biopesticides: a review of their actions, applications and efficacy. Pest Management Science 2000; 56:651-676.
36. Schmutterer H. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. Annual Review of Entomology 1990; 35:271-297
37. Law-Ogbomo KE. Reduction of post-harvest loss caused by *C. maculatus* (F) in three varieties of cowpea treated with plants oil. Journal of Entomology 2007; 4:194-201.
38. Boateng BA, Kusi F. Toxicity of Jatropha seed oil to *Callosobruchus maculatus* (Coleoptera: Bruchidae) and its parasitoid *Dinarmus basalis* (Hymenoptera: Pteromalidae). Journal of Applied Science Research 2008; 4:945-951.
39. Deng Y, Cao M, Shi D, Yin Z, Jia R, Xu J *et al*. Toxicological evaluation of neem (*Azadirachta indica*) oil: acute and subacute toxicity. Environmental Toxicology and Pharmacology 2013; 35:240-246.