Persistence of Cameroonian neem seed oil on *Callosobruchus maculatus* and *Sitophilus zeamais* and the degradation of its Azadirachtin A on treated maize and cowpea

Tofel, H.K.*[1,3], Nukenine, N.E.[2], Stähler, M.[1], Cornel, A.#[1]
[1]Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Institute for Ecochemistry, Plant Analysis and Stored Product Protection, Königin-Luise-Str. 19 D-14195 Berlin, Germany
[2]Department of Biological Sciences, University of Ngaoundere, P.O. Box 454 Ngaoundere, Cameroon
[3]Department of Biological Sciences, University of Bamenda, P.O. Box 39 Bamenda, Cameroon

*Corresponding author, Email: tofelhama@yahoo.fr
#Presenting author, Email: Cornel.Adler@jki.bund.de

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Abstract

The cowpea beetle *Callosobruchus maculatus* and the maize weevil *Sitophilus zeamais* are considered in many tropical countries as important pests of legumes and cereals. Botanicals are considered a good alternative to chemical insecticides, as they are often less toxic for users and more biodegradable. Products from the neem tree have long been used in many parts of the world for the control of various insect pests. The tree is common in North Cameroon. Therefore, it is an interesting source of insecticide for smallholder farmers. The degradation of Azadirachtin A in treated grain was determined with HPLC-MS between 0 and 180 days. Neem oil caused a significant day-dependent mortality of the insects and its effectiveness decreased with time. There was 100% mortality of *C. maculatus* (5 ml/kg) and *S. zeamais* (6 ml/kg) after treatment but for all dosages, there was less than 10% mortality 180 days after treatment. With 3 ml/kg, neem oil strongly inhibited the progeny production (100 %) of *C. maculatus* and *S. zeamais*. The tested oil was more persistent for inhibiting progeny production than on adult mortality. Azadirachtin A degraded slowly on treated grain from 1.31 mg/kg (0-day) to 0.31 mg/kg (180-day). The quality of treated grain could be examined at different storage times before recommending neem oil to smallholder farmers.

Keywords: persistence, Azadirachtin A, cowpea, maize, *Sitophilus zeamais*, *Callosobruchus maculatus*

1. Introduction

One of the remarkable plant studied by several researchers for its insecticidal and medicinal activities is *Azadirachta indica* A. Juss (Meliaceae) commonly called neem. The popularity of neem products increased day by day and this plant is known today as village pharmacy or plant of the 21st century (Ilesanmi and Gungula, 2013). Products from leaves, barks and seeds of this tree have been used for their medicinal properties (Nandagopal and Ghewande, 2004). Neem seed oil is used for soap manufacture (Schmutterer, 1990), motor lubricant and biodiesel (Anya et al., 2012) and an efficacious insecticide (Girish and Shankara, 2008). Barks and leaves of this plant are employed for the treatment of some diseases and are good antidotes against snake bite and scorpion sting (Yengué and Callot, 2002). The twigs of neem tree are used for dental hygiene (Agrawal, 2002). It 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).

To ensure food security for the whole year, farmers store more than 75% of their harvested cowpea and maize (Kumar, 1991) and therefore they have to treat these grains which are

heavily damaged respectively by the cowpea weevil *Callosobruchus maculatus* F. and the maize weevil *Sitophilus zeamais* Motschulsky. Labeyrie (1992) stated that without any protection farmers are working for insects. Tropical countries particularly those in Sub-saharan Africa are the world leaders in food insecurity (Babatunde et al., 2007; Ngamo and Hance, 2007; Birgit and Rosen, 2013). Therefore the fight against hunger and poverty needs to be intensified if this Millennium Development Goal is to be realized in Africa, Latin America and Asia. Neem oil is reported to protect stored products up to five or six months when applied at the rate of 1% (Guet, 2002). The high quantity oil applied to grains gives a bitter taste of products and therefore not accepted by local farmers. To promote the use of safer *A. indica* neem seed oil combined with good persistance in stored product protection with less than 1% of oil amount need to be reconsidered. Such studies could decipher the lower dosage of oil, and thus help growers to obtain more efficient plant-based insecticidal product for stored product protection with a minimized bitter effect. The main ingredient in neem products known for its efficacy against insect pests is Azadirachtin A (Schmutterer, 1995; Isman, 2006) which is extremely labile in light (Johnson et al., 2003) and have it potential efficacy within 10 and 12 days when used properly (Guet, 2002). Farmers treat and store their products for about one year. If the amount of the active ingredient is not enough to protect the treated grains after certain time, this could lead to the emergence of new insect pest in the stored products. Systematic scientific experimentation is necessary to determine the quantity of Azadirachtin A found in treated cowpea and maize and stored for different periods. The present study was thus carried out to evaluate the persistence of Cameroon neem oil on treated cowpea and maize against *C. maculatus* and *S. zeamais* respectively, as well as the degradation of Azadirachtin A on grains. This is the first study reporting the quantification of Azadirachtin A on treated grains with neem oil at diffirent storage periods.

2. Materials and Methods

2.1. 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), 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.

2.2. Origin of cowpea and maize

The maize variety was yellow Ricardino (KWS) harvested in an experimental field of Julius Kühn-Institut (JKI), Braunschweig, Germany in 2012. The organic cowpea (Black-eyed bean, Perou variety) was purchased in a tropical food store in Berlin, Germany.

2.3. Collection of Azadirachta indica seeds and oil extraction

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 dehusked and sun-dried. The drying temperature of the kernels was 34 ± 4°C. 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).

2.4. Adult toxicity test and F_1 progeny production

The volumes of 0.1, 0.15, 0.2, 0.25 and 3 ml of neem seed oil 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. To assess the persistence of the treatments (Obeng-Ofori and Amiteye, 2005), 20 adult beetles (*S. zeamais* or *C. maculatus*) were exposed to treated grain (maize or cowpea) which had been stored for 0, 15, 30, 60 and 180 days. Mortality counts were carried out 3 and 5 days after exposure for *C. maculatus* and *S. zeamais* 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. Insects were considered dead when no movement was observed after touching them carefully with forceps. After the 3-day and 5-day mortality recordings respectively for *C. maculatus* and *S. zeamais*, 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). To avoid generation overlaps, F_1 progeny were recorded 40 days and 50 days after infestation for cowpea and maize weevils respectively.

2.5. Azadirachtin A determination on treated grains

Similar dosages of each product as for the toxicity bioassay described above were used for this assay. Untreated grains were considered as control. A sample 5 g of grain was taken at 0, 1, 3, 7, 10, 14, 21, 30, 60, 90, 120, 150 and 180 days after treatment for azadirachtin A determination. The 5 g of cowpea or maize were weighed into a 50 ml polypropylene centrifuge tube and 100 µl of surrogate (Spinosyn A 100 g/l) were added. Extraction was performed by adding 25 ml acetone/water in proportion 80:20 v/v. The mixture was shaken using an ultrasonic bath for 15 min and then vortex-mixer for 45 min. An aliquot of 500 µl from the upper layer of extract was transferred to an Agilent vial and then dried to evaporate water. The extract was diluted with 1ml 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 dark at 4°C until analyze via LC/MS/MS. Each treatment was replicated four times and for each tube two replications was done for a total of eight 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 5µl. 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.

2.6. Data analysis

The mortality counts were corrected with Abbott's (1925) formula. Data on % cumulative corrected mortality and % reduction of progeny production were transformed to the arcsine [(square root(x/100)] and the number of progeny produced and the amount Azadirachtin A were 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).

3. Results

3.1. Persistence of A. indica seeds oil on adult C. maculatus and S. zeamais mortality

The results of the persistence of *A. indica* oil from sun-dried kernels showed that, the bioactivity of the oil decreased significantly ($P < 0.001$) with contents and storage time of treated grains (Table 1) At the lowest tested dose (2 ml/kg), 32.50% adult mortality of cowpea weevil was recorded 0-day storage while after 180 days no adult mortality was observed. At the highest dose (6 ml/kg) 15 days after storage of treated grains adult mortality of cowpea weevil decreased from 100% to 38.29% and no adult mortality was observed when the treated grains were infested after 180 days. Except the dose level of 5 ml/kg, the mortality of *S. zeamais* 60 days after treatment of maize did not differ from mortality at 0 day ($P > 0.05$) but drastically decreased after 180 days. Neem oil was more persistent on maize than on cowpea. Only after 0-day and at the highest dose (6 ml/kg) did the oil achieve 100% adult mortality of *C. maculatus*. In *S. zeamais* the 100% mortality remained up to 60 days.

Table 1 Corrected cumulative mortality of *Callosobruchus maculatus* and *Sitophilus zeamais* exposed in grains treated with neem seed oil after different periods of storage.

Insects /doses (ml/kg)	Infestation period (days)/ % mean mortality†					
	0	15	30	60	180	$F_{(4, 15)}$‡
C. maculatus						
0	0.00 ± 0.00^{e}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00	
2	32.50 ± 1.44^{dA}	9.01 ± 2.53^{bB}	1.32 ± 1.32^{cC}	5.00 ± 2.04^{abcBC}	0.00 ± 0.00^{C}	28.41***
3	55.00 ± 5.40^{cA}	26.91 ± 3.08^{aB}	11.52 ± 1.17^{bC}	2.50 ± 1.14^{bcD}	0.00 ± 0.00^{D}	64.04***
4	77.50 ± 4.79^{bA}	24.15 ± 5.38^{aB}	23.03 ± 4.89^{abB}	7.50 ± 1.44^{abC}	0.00 ± 0.00^{D}	62.12***
5	95.00 ± 2.04^{aA}	37.11 ± 5.46^{aB}	28.16 ± 6.22^{abB}	10.00 ± 2.04^{aC}	0.00 ± 0.00^{D}	93.98***
6	100 ± 0.00^{aA}	38.29 ± 4.38^{aB}	34.48 ± 5.29^{aB}	10.00 ± 2.04^{aC}	0.00 ± 0.00^{D}	275.71***
$F_{(5, 18)}$	172.87***	30.57***	27.10***	7.92***	–	
S. zeamais						
0	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{f}	0.00 ± 0.00^{d}	0.00 ± 0.00	
2	13.75 ± 2.39^{dA}	26.25 ± 6.88^{cA}	30.00 ± 4.46^{eA}	26.25 ± 7.47^{cA}	1.25 ± 1.25^{B}	9.39***
3	52.50 ± 7.22^{cA}	57.50 ± 6.01^{bA}	48.00 ± 6.25^{dA}	35.00 ± 8.42^{cA}	0.00 ± 0.00^{B}	27.76***
4	78.75 ± 3.75^{bA}	91.25 ± 5.15^{aA}	77.50 ± 3.23^{cA}	91.25 ± 4.27^{abA}	5.00 ± 3.54^{B}	31.37***
5	100 ± 0.00^{aA}	100 ± 0.00^{aA}	91.25 ± 1.25^{bB}	88.75 ± 3.75^{bB}	5.00 ± 2.89^{C}	131.39***
6	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	6.25 ± 2.39^{B}	318.13***
$F_{(5, 18)}$	273.33**	89.01***	214.45**	67.03***	1.89^{ns}	

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

3.2. Persistence of A. indica seeds oil on C. maculatus and S. zeamais F_1 progeny production

In all evaluated treatments, the application of *A. indica* seed oils suppressed F_1 progeny emergence in *C. maculatus* and *S .zeamais*, regardless of storage time of treated grains (Table 2). When treated with 2 ml/kg of the *A. indica* seed oils, both cowpea and maize grains registered progeny production but in few number compared to the control, although the number of the offspring increased with storage time in cowpea (0.25 at 0-day to 136.25 at 180 days) than in maize (4.25 at 0-day to 13.25 at 180 days). No adult emergence was observed in both insect species when grains were treated with ≥ 3 ml/kg neem seed oil independently of exposure time of the treated grains.

Table 2 Progeny production of *Callosobruchus maculatus* and *Sitophilus zeamais* exposed to grains treated with neem seed oil after different periods of storage.

Insects /doses (ml/kg)	Infestation period (days)/ % mean mortality†					
	0	15	30	60	180	$F_{(4, 15)}$‡
C. maculatus						
0	472.75± 13.39^{a}	473.00 ± 16.14^{a}	467.75 ± 17.36^{a}	412.49 ± 18.43^{a}	418.00 ± 11.51^{a}	0.84^{ns}
2	0.25 ± 0.25^{bD}	9.00 ± 1.58^{bC}	10.75 ± 1.18^{bC}	21.75 ± 1.93^{bB}	136.25 ± 3.90^{bA}	364***
3	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	–
4	0.00 ± 0.00 ^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	–
5	0.00 ± 0.00 ^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	–
6	0.00 ± 0.00 ^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	–
$F_{(5, 18)}$	1245.11***	844.51***	716.71***	485.49***	1155.19***	
S. zeamais						
0	47.00 ± 1.87^{a}	55.25 ± 2.95^{a}	56.00 ± 0.00^{a}	53.75 ± 3.01^{a}	54.75 ± 4.21^{a}	0.91^{ns}
2	4.25 ± 2.02^{b}	6.75 ± 1.11^{b}	8.25 ± 0.63^{b}	10.25 ± 0.75^{b}	13.25 ± 0.63^{bA}	0.48^{ns}
3	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	–
4	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	–
5	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	–
6	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	–
$F_{(5, 18)}$	283.93***	293.13 ***	345.88***	282.84***	156.18***	

†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

3.3. Degradation of Azadirachtin A on treated cowpea and maize with neem oil at different periods of storage

The result of the degradation on Azadirachtin A contained in neem oil on treated cowpea and maize (Figure 1) showed that the active ingredient in neem oil known for its efficacy against insect pests was affected by exposure periods ($P < 0.001$). The data indicated that Azadirachtin A was relatively stable on maize 21 days after treatment with content varying from 1.30 mg/kg (0 day) to 1.28 mg/kg (21 days) when treated with 6 ml/kg neem oil while on cowpea after 14 days less than 1 mg/kg of Azadirachtin A was estimated. After 180 days, maize treated with lowest dose of 2 ml/kg neem oil showed that 0.10 mg/kg of Azadirachtin A remained on maize. Similar trend was observed on cowpea at the same dosage.

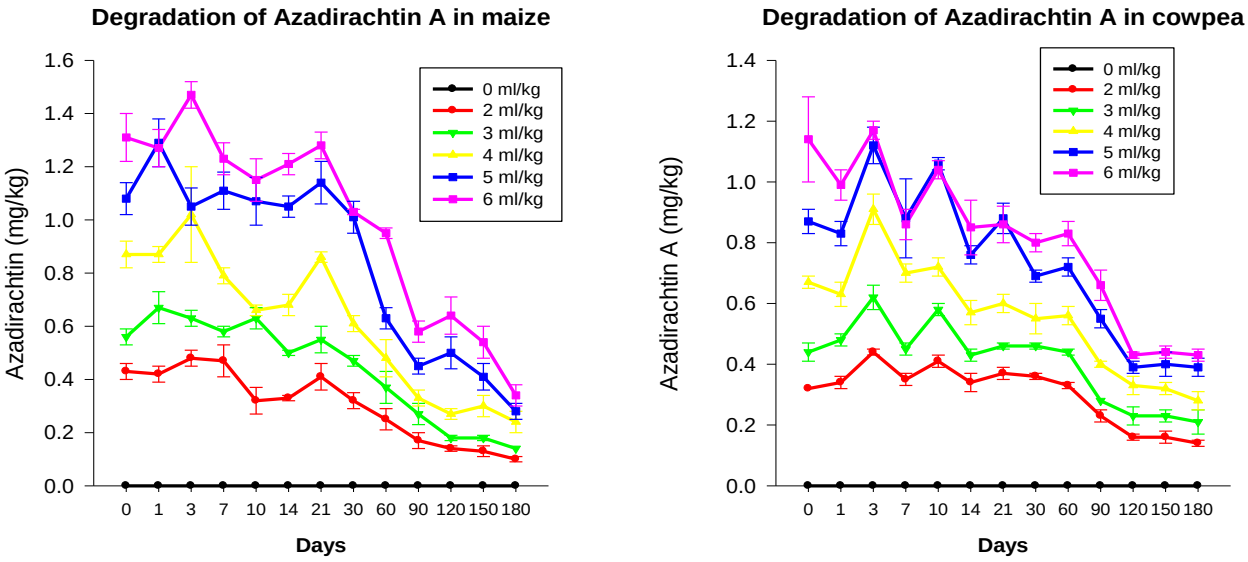

Figure 1 Degradation of Azadirachtin A in maize and cowpea treated with neem oil after different periods of storage.

4. Discussion

This study has shown that *C. maculatus* and *S. zeamais* adult mortality increase with increasing dose and time post exposure. Within 0-day after infestation, 100% mortality were recorded respectively for *C. maculatus* (6 ml/kg) and *S. zeamais* (5 ml/kg) This trend is consistent with those reported by Mbaiguinam et al. (2006) as they used *A. indica* seed oil from Chad on *C. maculatus* and Obeng-Ofori and Amiteye (2005) on *S. zeamais* as different vegetable oils were applied. *C. maculatus* was less susceptible than *S. zeamais* and this was remarkable from the 15th day after infestation. Persistency of neem oil declined from 100% after treatment to 0% mortality 180 days storage on *C. maculatus* while the efficacy of the oil stayed closely the same on *S. zeamais* 60 days and decreased to less than 10% after 180 days of storage. It becomes more likely that the mortality of *C. maculatus* was due more to physical rather than chemical effects, since when the storage period increased, adult mortality also decreased with no adult mortality recorded after 180 days of storage. Oil, when is coated to insect body may cover the cuticle of insect, which will in turn prevent respiration, leading to the death of the insect (Don-Pedro 1989; Iloba and Ekrakene 2006).This view is supported by the work of Don-Pedro (1989) using vegetable oils against *S. zeamais* on wheat. In his study, grains treated with groundnut oil resulted in mortality of up to 30% at the dose of 17.5 ml/kg, but its toxicity was not appreciable after 14 days. The physical action of vegetable oils is based on oil quantity. Raguraman and Singh (1997) reported that low concentration of neem oil produced negligible mortality after one and two days of treatment. This difference could be related to the coat of the treated grains. Cowpea seed coat was thinner and permitted oil to infiltrate into the seed than that of maize which was more consistent and maintained it on grains. Through this mechanism of permeability, the physical contact between insect and oil is reduced and limited *C maculatus* mortality by anoxia. More so *S. zeamais* adult feed on grain, during food intake took some triterpenoid compounds of neem oil which could turn to the death of adult insect. Similar result was registered with *Jatropha* seed oil on cowpea within 60 days same period of storage by Boateng and Kusi (2008).

Stomach poisoning properties may be also part of the cause of the death difference in insect species. Adult *C. maculatus* do not feed on cowpea while adult *S. zeamais* adult feed on

maize. This means while feeding maize weevils took some quantity of oils which contained insecticidal compounds (Debashri and Tamal, 2013) and death ensues. The richness of neem oil in limonoids constituents like azadirachtin, nimbin, salanin, nimbidin and meliantriol (Schumutterer, 1990; Addea-Mensah, 1998), reduces insects feeding which consists in the input from receptors that normally respond to phagostimulants, or from stimulation of specific deterrent cells or both (Schmutterer, 1990; Petit, 2008) and through a reduction in food intake due to the bitter taste and the bad odour of neem oil occurred by nimbidin (Rukmini, 1987). Thus, may explain the death of *S. zeamais* at least in part by starvation.

Results of inhibition of the progeny emergence showed that neem oil reduced significantly progeny emergence of *C. maculatus* and *S. zeamais* showing their ability to control both insects. Except for grains treated with the lowest dose 2 ml/kg, no insect emerged means that the neem oil acted as physical barrier on egg hatching or chemically on immature stages depending on insect species. Suppression of emergence in *C. maculatus* could be related to physical action of the neem seed oil. This cowpea beetle laid eggs on the seed, and as the seeds have been covered with oil, female had not or less ability to coat the eggs on the seeds. Therefore is not possible for the eggs to hatch into the grains. The similar effect was reported by others researchers where neem oil inhibited completely the progeny production of *S. oryzae* and *C. maculatus* (Bamaiyi et al., 2007; Ilesanmi and Gundula 2013). Neem oil like other vegetable oils has the ability to penetrate the chorion of eggs via the micropyle and oil might occlude egg funnel which block exchange with outside leading to the asphyxiation of developing insect (Copping and Menn, 2000).

More than mechanical action of oils attributed neem oil to inhibit progeny production, its specificity could be found in *S. zeamais*. Female maize weevil laid eggs into grains. If, on treated grains oviposition is not deterred by the presence of oil, the development of immature stages is affected chemically. As the oil has ability to infiltrate the grains, larvae and nymphs of *S. zeamais* fed inside the grains and digested some quantity of azadirachtin which were present on treated grains after 180 days. This compound has growth regulatory effects which may block the developmental stages of the weevils or cause mortality of immature stages (Isman, 2006). Udo (2005) stated that, there is relationship between F_1 progeny emergence and adult mortality. But it was not the case in the present work, since there were living *S. zeamais* and *C. maculatus* after different periods of infestation and no offspring was recorded at the dosage level $\geq$ 3 ml/kg. Most of time the efficacy of botanicals is evaluated by its efficacy to kill adult insects but this present study showed that inhibition of progeny production has equal or more value than adult mortality. It better to have stored products with the presence of insect pests that are unable to procreate than having products with killed parent insects but which could not stop the development of new generations.

Many factors as the pH, temperature, relative humidity, daylight, the ultra violet lights and the carriers (Barreck et al., 2004; El Shafie et al., 2012) affect the degradation of Azadirachtin A. Barrack et al. (2004) reported that the disappearance of Azadirachtin A in daylight was faster than in dark. Radwan and El-Shiekh (2012) stated that some neem formulation retain their Azadirachtin A content for at least one year when stored in dark. Unfortulaly no study was carried out before to evaluate the degradation of Azadirachtin A contained in neem oil on treated maize and cowpea. Nevertheless the result of the present study showed that on treated grains, the main component in neem oil known for its insecticidal activities degraded slowly and reduced four times within six months of storage in dark.

5. Conclusions

The results of our study showed that for all doses, mortality declined after 180 days of storage to 0% for *C. maculatus* and about 5% for *S. zeamais.* Only 3 ml/kg (0.3%) of neem oil is sufficient to inhibit the emergence of the progeny up to six months after treatment. Also neem seed oil persisted more in suppression of progeny production compared to adult mortality. Azadirachtin A, the active substances in neem oil degraded gradually on treated grains and was less stable after six months on maize and cowpea. For proper sound while consuming grains treated with neem oil, further studies, should be investigated for the quality and taste of the treated grains and assessments at the farmer's level will be done.

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