

## RESEARCH

## Efficacy of *Plectranthus glandulosus* (Lamiaceae) and *Callistemon rigidus* (Myrtaceae) Leaf Extract Fractions to *Callosobruchus maculatus* (Coleoptera: Bruchidae)

Simon Pierre Yinyang Danga,<sup>1,2</sup> Elias Nchiwan Nukenine,<sup>1</sup> Lame Younoussa,<sup>1</sup> Cornel Adler,<sup>3</sup> and Charles Okechukwu Esimone<sup>4</sup>

<sup>1</sup>Department of Biological Sciences, Faculty of Science, University of Ngaoundere, PO Box 454, Ngaoundere, Cameroon

<sup>2</sup>Corresponding author, e-mail: dangayisipi@yahoo.fr

<sup>3</sup>Federal Biological Research Centre for Agriculture and Forestry (BBA), Institute of Stored Product Protection, Königin-Luise-Str. 19, D-14195 Berlin, Germany

<sup>4</sup>Department of Pharmaceutical Microbiology & Biotechnology, Faculty of Pharmaceutical Sciences, Agulu, Nnamdi Azikiwe University, PMB 5025, Awka, Anambra State, Nigeria

**Subject Editor:** Christos Athanassiou

J. Insect Sci. (2015) 15(1): 139; DOI: 10.1093/jisesa/iev117

**ABSTRACT.** As part of on-going efforts to use eco-friendly alternatives to chemical pesticides, methanol crude extracts of *Plectranthus glandulosus* and *Callistemon rigidus* leaves were sequentially fractionated in hexane, chloroform, ethyl acetate, and methanol to establish the most active fraction(s) against *Callosobruchus maculatus* in cowpea. Cowpea seeds (25 g) were treated with 0.5, 1, 2, and 4 g/kg of extract to evaluate the contact toxicity and F<sub>1</sub> progeny production of the beetles in the laboratory. Mortality was recorded 1, 3, and 7 d postexposure. *P. glandulosus* hexane fraction was more toxic than the other fractions recording 100% mortality at 4 g/kg, within 7 d with LC<sub>50</sub> of 0.39 g/kg. Hexane fraction of *C. rigidus* showed superior toxicity, causing 100% mortality at 4 g/kg within only 1 d of exposure with LC<sub>50</sub> of 1.02 g/kg. All the fractions greatly reduced progeny emergence, with *C. rigidus* hexane fraction being the best progeny inhibitor. Fractions of *P. glandulosus* and *C. rigidus* leaves had sufficient efficacy to be a component of storage pest management package for *C. maculatus*.

**Key Words:** *Plectranthus glandulosus*, *Callistemon rigidus*, fraction, *Callosobruchus maculatus*, contact toxicity

The aim of agriculture is high yield to provide food for the population. One of the most important constraints of having every day sufficient food is the postharvests preservation of its quality and quantity (Olufunmilayo 2012). Unfortunately, increasing the supply of the crop is marred by losses during storage caused principally by insects (Ngamo et al. 2007b). Among these insects, *Callosobruchus maculatus* F. also called cowpea beetle, cowpea weevil, or bruchid is one of the most destructive cowpea seeds starting from the field just before harvest, and the insect is carried into the store where population builds up rapidly (Ngamo et al. 2007a). This weevil has caused losses both in quantity and quality of the stored seeds. Estimates of storage losses are highly variable ranging widely from 4 to 90% (Umeozor 2005) due to perforations by this weevil, thus reducing the degree of usefulness and making the seeds unfit either for planting or for human consumption (Ali et al. 2004).

In tropical countries, the cowpea *Vigna unguiculata* (L.) Walp (Fabaceae) is an important source of proteins in diet of people that cannot afford protein foods such as meat and fish (Oluwafemi et al. 2013). The dry seed consists of about 25% protein and 67% carbohydrate. It is also a good source of calcium, iron, vitamins, and carotene (Olufunmilayo 2012). Cowpea is favored by farmers because of its ability to maintain soil fertility, income, its use as animal fodder, and comparably high yields in harsh environment where other food legumes do not thrive (Oluwafemi et al. 2013). Cowpea feeds millions of people in developing countries like Cameroon with an annual world-wide production estimated around 4.5 metric tons on 12–14 million ha (Diouf 2011). In Cameroon, the dry seeds are prepared as porridge and also as traditional steamed cake called “khoki” wrapped in leaves (Ntonifor et al. 2010). In Northern Cameroon, 78% of farmers produce cowpea, which is highly susceptible to insect attacks, particularly of the weevil *C. maculatus* (Ngamo et al. 2007a). Many methods have been used to prevent these post harvest losses (Kamanula et al. 2010). To prevent the loss of crops during storage and on field, products usually rely on chemical insecticides. Although they are still the most effective, their

repeated use caused environmental pollution, development of resistant pest populations, and residues on treated crops or seeds resulting in adverse effects on human health (Devine and Furlong 2007, Hong et al. 2013). To address these shortcomings of synthetic insecticides, there has been a great deal of interest to seek alternative control methods that are less toxic to nontarget organisms and are biodegradable (Isman 2006, Stevenson et al. 2012).

Peasant farmers and researchers often claim successful use of plant materials in stored products insect pest control including plant extracts, vegetable oils, spices, and plant powder (Isman 2006). Adedire and Akinneye (2003) reported that some plant materials and local traditional methods have less harmful nature to environment and nontargeted organisms than chemical insecticides and suggested that their use needed exploitation. In this context, neem trees (*Azadirachta indica* A. Juss [Meliaceae]) are grown commercially in plantations to produce azadirachtin, a chemical extracted from the seeds and leaves. Azadirachtin has been promoted as a new insecticide that is considered more “environmentally friendly” than synthetic insecticides (Csurhes 2008). Furthermore, Wiesbrook (2004) also reported that Neem has an extremely low mammalian toxicity and is least toxic of the botanical insecticides, with an LD<sub>50</sub> of 13,000 mg/kg.

*Plectranthus glandulosus* Hook f (Lamiaceae) is one of 18 species of *Plectranthus* genus, which are indexed in the world (Abdel-Mogib et al. 2002). In Cameroon, *P. glandulosus* is known as medicinal plant used against influenza, cough, and chest complaints (Oliver-Bever 1982). It is a plant whose leaves are commonly used to protect stored grains (Nukenine et al. 2007, 2010, 2011b, 2013; Tofel et al. 2014). Earlier, the fractions of the plant’s leaves showed strong mosquito larvicidal activity against *Aedes aegypti* L. (1762), *Anopheles gambiae* Giles (1902), and *Culex quinquefasciatus* Say (1823) vector mosquitoes (Danga et al. 2014c). The leaf essential oil of the same plant has been found very effective against larvae and pupae of *Ae. aegypti*, *An. gambiae*, and *Cx. quinquefasciatus* vector mosquitoes (Danga et al. 2014b).

*Callistemon rigidus* (Myrtaceae) is a stiff and upright shrub characterized by red flower spikes that are shaped like bottlebrushes (Danga et al. 2014b). In Cameroon, *C. rigidus* is known in folk medicine for its anticough and antibronchitis effects. Essential oil extracted from the leaf has been found to be effective against fourth-instar larvae and early pupae of *Ae. aegypti*, *An. gambiae*, and *Cx. quinquefasciatus* vector mosquitoes (Danga et al. 2014b).

In recent studies, Pangnakorn (2009) reported that hexane, chloroform, and methanol fractions from *Vetiver zizanioides* L. (Poaceae) were found to be toxic to *C. maculatus*. In the same vein, Epidi and Udo (2009) indicated that hexane, chloroform, ethyl acetate, and butanol fractions of *Dracaena arborea* Hort. Angl. ex Link (Dracaenaceae) showed protectant activities against adult *C. maculatus*. It was based on this view that, an investigation of toxicity and progeny inhibition effects of four solvent extract fractions from *P. glandulosus* and *C. rigidus* leaves has been carried out to establish the most active one(s) against adult *C. maculatus*. Essential oil and powder from *P. glandulosus* leaves have been used to protect stored grains from the attack of pests (Nukenine et al. 2007, 2010, 2011b, 2013; Tofel et al. 2014). However, this is the first report the leaves of both plants are fractionated and tested against beans beetle, *C. maculatus*. Both plants have been chosen based on their large availability and fractionated to find the solvent that will extract the most active compounds.

## Materials and Methods

**Collection of Plant Materials.** The fresh leaves of *P. glandulosus* and *C. rigidus* were collected in October 2011 (8:00 am–12:00 pm Cameroon time) in Ngaoundere (latitude 7° 22' N and longitude 13° 34' E, altitude of 1,100 masl), located in the Adamawa region (plateau), Cameroon. Plants were <1-yr old, and only the green leaves were harvested. They were identified for confirmation at the National Herbarium of Cameroon, where voucher specimens were deposited with the following voucher number: 18564/SRF/CAM and 41168HCN for *C. rigidus* and *P. glandulosus*, respectively. Leaves were dried at room (temperature 25 ± 3°C and relative humidity of 81 ± 2%) before thorough washing with distilled water and then ground in powdered form using mini electric grinding mill (Alvan Blanch, Chelworth Malmesbury Wiltshire, England) until the powder passed through a 0.4-mm mesh sieve. The powder was stored in opaque containers inside a refrigerator at -4°C and transported by road in February 2012 to Faculty of Pharmaceutical Sciences, Agulu, Anambra state, Nigeria and Nnamdi Azikiwe University, Awka; Anambra state, Nigeria, where the experiments were carried out and then stored in a refrigerator at -4°C until needed.

**Extraction and Fractionation of Plant Materials.** The extraction scheme was performed according to the method adopted by Okoye and Osadede (2009). From the collection of plant material powder, 700 g were extracted for 3 d by cold maceration in 1.5 liters of methanol (JQ American Corporation JQLAB, CA), stirring it thrice per day (morning, noon, and afternoon) in the laboratory of Pharmaceutical and Medicinal Chemistry. The maceration process was then repeated thrice for maximal extraction. The methanol crude extract was then collected and concentrated almost to dryness under vacuum at 40°C using rotary evaporator RE300 ROTAFLO (Fisher Scientific UK Ltd., UK). The methanol crude extract was first absorbed on silica gel (60–200 mesh size) (GeeJay Chemicals Ltd., UK) and sequentially fractionated in 1 liter of hexane, chloroform, ethyl acetate, and methanol (JQ American Corporation JQLAB, CA) in increasing order of polarity. All the fractions so obtained were filtered many times adding fresh solvent until clear phase was obtained before passing to the next solvent using Whatman No. 1 filter paper (size: 24 cm, UK) (Cole-Parmer Instrument Co. Ltd., UK). The same rotary evaporator was used to concentrate the fractions at 40 ± 5°C. The yields were *P. glandulosus* (9.96, 10.94, 13.68, 12.91, and 32.91%) and *C. rigidus* (27.28, 8.18, 11.35, 12.42 and 37.62%) for methanol crude extract, hexane, chloroform, ethyl acetate, and methanol fractions, respectively. The crude extracts and

fractions were stored in refrigerator (American refrigeration Company, Inc.) at -4°C until needed.

**Rearing of Insect.** Rearing process was adopted in conjunction with Suleiman et al. (2012) method with slight modifications. The cowpea seed beetle, *C. Maculatus*, used to establish the insect culture was obtained from infested cowpea seeds purchased locally from a store at Awka market, Anambra State, Nigeria, and the culture of *C. maculatus* was raised in the laboratory. Local variety called “Black eye beans” was used both for culture and bioassays. Damaged grains were removed, and only clean beans were put in plastic jars and disinfested in a refrigerator at -4°C for 3 d. After disinfestations, grains were kept in the laboratory for acclimation 5 d prior to the culture process and toxicity bioassay. The clean white cowpea seeds (200 g) were placed in five plastic jars, and 100 unsexed *C. maculatus* were introduced in each plastic jar. The plastic jars were first covered with muslin and secured with their lids (pierced for air). The insects were allowed to oviposition for 5 d and then sieved out. The infested seeds were placed in the laboratory at ambient temperature and observed daily for emergence of adults. This culture was maintained at 28 ± 3°C, 81 ± 4% RH, and under a photoperiod of 12:12 (L:D) h and used as source of *C. maculatus*.

**Adult Toxicity Tests and Progeny Production.** Adult toxicity tests and progeny production were conducted according to the method of Nukenine et al. (2011a). The application rates were 0.5, 1, 2, and 4 g/kg. These rates were obtained by adding 0.0125, 0.025, 0.05, and 0.1 g of crude extract or fraction to 25 g of white beans in a 500 ml plastic jar. The extracts were accurately weighted on Adventurer Scale (Ohaus Corp. Pine Brook, NJ; max cap: 210 g; readability: 0.0001 g). The doses were defined after a preliminary test. Each dose of the crude extracts and fractions was diluted in 1.5 ml of acetone (99% purity) (JQ American Corporation JQLAB, CA). Jars containing test solutions and 25-g samples of grain were gently hand shaken for 5 min to get uniform coating. The samples were kept for 2 h to allow the solvent to evaporate before introducing the cowpea beetles. Twenty (10 males and 10 females) weevils (1–2-d old) were introduced into each plastic jar (Ojo et al. 2013). Only active beetles were used. Negative controls contained beans mixed with 1.5 ml of acetone alone without any trace of insecticide. Malagrain DP 5 (5% Malathion) was purchased from an agric-products shop at Ngaoundere, Cameroon, and used as positive control. In the treated control, 12.5 mg of Malagrain was introduced in 25 g of beans (0.5 g/kg, recommended dose). The treatments were arranged in a completely randomized design on shelves with three replications per treatment. Each treatment was repeated three times. All treatments were maintained in the laboratory under ambient conditions. The daily temperature and relative humidity in the laboratory ranged from 23 to 29°C and 61 to 92% RH, respectively. Mortality was recorded 1, 3, and 7 d after beetles' infestation. Knock-down insects were not counted. The beetles were considered as dead, if they were not responsive to a gentle prodding with a fine needle. There was no recovery of insects that were removed from the plant materials.

On the 7th day, all insects were removed, and the number of dead and alive beetles was recorded. All treatments began on the same day and, therefore, were exposed to the same temperature and relative humidity regime. The counting of F<sub>1</sub> progeny was carried out daily for 11 d, commencing 25 d after infestation. After each counting session, the insects were removed from the jars and recorded.

**Data Analysis.** Data on % mortality, including control mortality, and % reduction were tested for normality and heterogeneity of variance and then subjected to analysis of variance procedure using Statistical Package for Social Science (SPSS v.17.0) for Windows software (SPSS Inc., Ltd., Quarry Bay, Hong Kong). The same software was used for figures. The Student–Newman–Keuls test at  $P=0.05$  was used for mean separation. Probit analysis (Finney 1971) was applied to determine lethal dosages causing 50% (LC<sub>50</sub>) mortality of adult *C. maculatus* at 1, 3, and 7 d posttreatment. Abbott's formula (Abbott 1925) was

**Table 1. Corrected mortality of adult *C. maculatus* exposed to different solvent leaf extract fractions of *P. glandulosus* in the laboratory**

Periods of exposure (d)	Doses (g/kg)	Methanol crude extract	Hexane fraction	Chloroform fraction	Ethyl acetate fraction	Methanol fraction	F
1	0 (ctrl)	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	—
	0.5	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	—
	1	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	—
	2	0 ± 0 <sup>aB</sup>	0 ± 0 <sup>aB</sup>	8.3 ± 3.3 <sup>aA</sup>	8.3 ± 1.6 <sup>7aA</sup>	0 ± 0 <sup>aB</sup>	79*
	4	11.7 ± 1.7 <sup>bC</sup>	11.7 ± 1.7 <sup>bC</sup>	28.3 ± 3.3 <sup>bA</sup>	21.7 ± 4.4 <sup>bB</sup>	6.7 ± 1.6 <sup>bD</sup>	102*
	Malag.	100 ± 0 <sup>c</sup>	100 ± 0 <sup>c</sup>	100 ± 0 <sup>c</sup>	100 ± 0 <sup>c</sup>	100 ± 0 <sup>c</sup>	—
3	0 (ctrl)	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	—
	0.5	0 ± 0 <sup>aC</sup>	10 ± 2.8 <sup>bA</sup>	0 ± 0 <sup>aC</sup>	6.7 ± 1.6 <sup>bB</sup>	0 ± 0 <sup>aC</sup>	191*
	1	16.7 ± 1.6 <sup>bC</sup>	31.7 ± 1.6 <sup>cA</sup>	11.7 ± 3.3 <sup>bD</sup>	20 ± 2.8 <sup>cB</sup>	8.3 ± 1.6 <sup>aE</sup>	64*
	2	43.3 ± 3.3 <sup>cC</sup>	71.7 ± 4.4 <sup>dA</sup>	41.7 ± 4.4 <sup>cC</sup>	60 ± 5.7 <sup>dB</sup>	28.3 ± 3.3 <sup>bD</sup>	85*
	4	78.3 ± 3.3 <sup>dC</sup>	98.3 ± 1.7 <sup>eA</sup>	73.3 ± 4.4 <sup>dD</sup>	90 ± 5.7 <sup>eB</sup>	73.3 ± 4.4 <sup>cD</sup>	52*
	Malag.	100 ± 0 <sup>e</sup>	100 ± 0 <sup>e</sup>	100 ± 0 <sup>e</sup>	100 ± 0 <sup>e</sup>	100 ± 0 <sup>d</sup>	—
7	0 (ctrl)	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	—
	0.5	11.7 ± 1.6 <sup>bD</sup>	28.3 ± 3.3 <sup>bB</sup>	11.7 ± 3.3 <sup>bD</sup>	41.7 ± 3.3 <sup>bA</sup>	18.3 ± 6 <sup>bC</sup>	97*
	1	38.3 ± 6 <sup>cC</sup>	76.7 ± 4.4 <sup>cA</sup>	21.7 ± 3.3 <sup>bD</sup>	63.3 ± 4.4 <sup>cB</sup>	36.7 ± 4.4 <sup>cC</sup>	78*
	2	78.3 ± 4.4 <sup>dC</sup>	96.7 ± 3.3 <sup>dA</sup>	55 ± 5 <sup>cE</sup>	81.7 ± 1.6 <sup>dB</sup>	73.3 ± 4.4 <sup>dD</sup>	59*
	4	100 ± 0 <sup>eA</sup>	100 ± 0 <sup>dA</sup>	88.3 ± 4.4 <sup>dC</sup>	96.7 ± 1.6 <sup>eB</sup>	96.7 ± 1.6 <sup>eB</sup>	41*
	Malag.	100 ± 0 <sup>e</sup>	100 ± 0 <sup>d</sup>	100 ± 0 <sup>e</sup>	100 ± 0 <sup>e</sup>	100 ± 0 <sup>e</sup>	—
F		107.6*	104.8*	72.6*	62.5*	64.2*	

Means within a column, comparing the different concentrations of a fraction (small letters) and within a line, comparing the different fractions at a single concentration (capital letters) followed by the same letter do not differ significantly at  $P = 0.05$  (Student–Newman–Keuls's test)

\* $P < 0.01$ ; all cases  $df = 3, 15$ .

used to correct for control mortality before Probit analysis and analysis of variance.

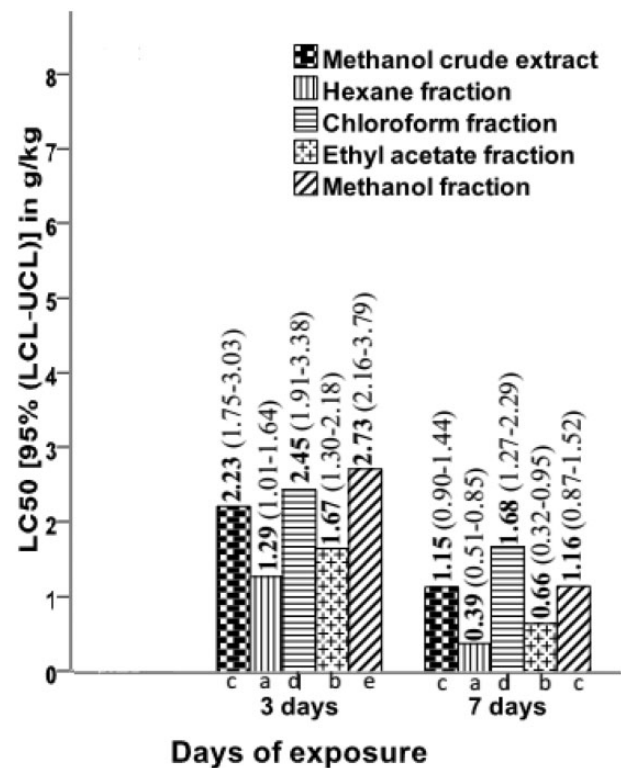
## Results

**Insecticidal Activity.** The results of the *P. glandulosus* toxicity showed that the methanol crude extract and the four solvent fractions caused significant mortality to adult *C. maculatus* (Table 1). Mortality increased with dose level and time postexposure for all the insecticidal materials. In general, hexane fraction was more toxic to the cowpea beetle than the other solvent fractions. At the highest dose of 4 g/kg, hexane fraction caused 100% mortality to adult beetles within 7 d of exposure. The methanol crude extract achieved the same mortality rate and at the same period of exposure. The  $LC_{50}$  values were 0.39, 0.66, and 1.15 g/kg for hexane fraction, ethyl acetate fraction, and methanol crude extract, respectively (Fig. 1). The results of the *C. rigidus* toxicity tests summarized in Table 2 showed the potential toxicity of hexane fraction causing 100% mortality to adult cowpea beetles at the highest dose of 4 g/kg within only 1 d of exposure. It registered  $LC_{50}$  value of as low as 1.02 (Fig. 2). Chloroform and ethyl acetate fractions equally obtained 100% mortality at the highest dose of 4 g/kg 7 d post exposure with  $LC_{50}$  values of 1.11 and 1.73 g/kg, respectively.

**Progeny Production.** The crude extract and fractions of *P. glandulosus* globally caused significant reduction in progeny production relative to the control, which was dose dependent (Table 3). The lowest dose of 0.5 g/kg roughly caused >80% suppression of  $F_1$  progeny emergence. Hexane and chloroform fractions achieved complete suppression of progeny emergence from 2 g/kg dose. As for *C. rigidus*, hexane fraction still caused total inhibition of  $F_1$  progeny emergence. No progeny emergence was noticed at all doses (Table 3). Crude extract and chloroform fraction at 2 g/kg and ethyl acetate fraction at 1 g/kg equally caused 100% suppression of progeny emergence.

## Discussion

Plant extract fractionation is the first route of developing an active component for desired effect. After the most active fraction is found, the next step is the bio-guided fractionation process, which leads to the identification of the active principle (Magadula et al. 2009). In this context, methanol crude extract of Cameroonian *P. glandulosus* and *C. rigidus* have been sequentially fractionated using hexane, chloroform, ethyl acetate, and methanol. The results showed that both crude



**Fig. 1.** Probit analysis of different solvent leaf fractions of *P. glandulosus* in the laboratory at 1, 3, and 7 d after treatment against adult *C. maculatus*.

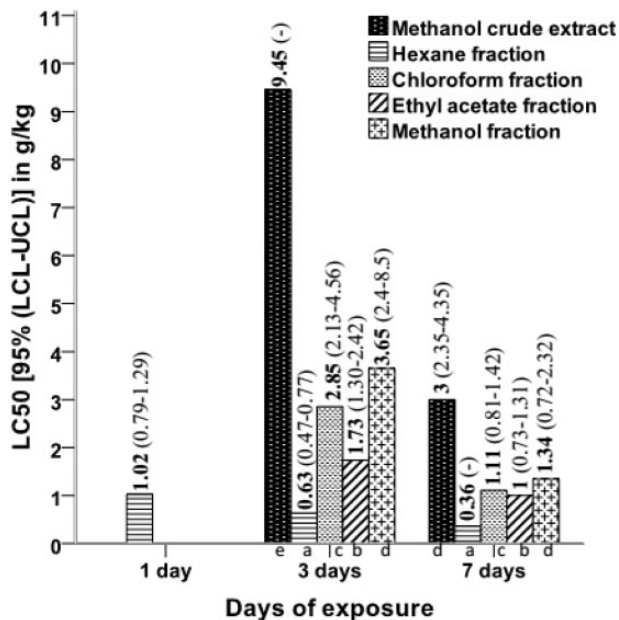
extracts and fractions of *P. glandulosus* and *C. rigidus* caused high mortality of adult *C. maculatus* in treated cowpea seeds. Some researchers who had earlier evaluated plant extracts as botanical insecticides and grain protectants had found them to be effective against storage beetles (Isman 1999). In this study, hexane fractions of both plants appeared to be more toxic than the other solvent fractions tested. The results from this investigation are similar to the findings of Adedire et al. (2011) who obtained 100% mortality of adult *C. maculatus* in cowpea seeds

**Table 2. Corrected mortality of adult *C. maculatus* exposed to different solvent leaf extract fractions of *C. rigidus* in the laboratory**

Periods of exposure (d)	Doses (g/kg)	Methanol crude extract	Hexane fraction	Chloroform fraction	Ethyl acetate fraction	Methanol fraction	F
1	0 (ctrl)	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	—
	0.5	0 ± 0 <sup>aB</sup>	18.3 ± 1.6 <sup>bA</sup>	0 ± 0 <sup>aB</sup>	0 ± 0 <sup>aB</sup>	0 ± 0 <sup>aB</sup>	68*
	1	0 ± 0 <sup>aB</sup>	38.3 ± 3.3 <sup>cA</sup>	0 ± 0 <sup>aB</sup>	0 ± 0 <sup>aB</sup>	0 ± 0 <sup>aB</sup>	91*
	2	0 ± 0 <sup>aB</sup>	86.7 ± 4.4 <sup>dA</sup>	0 ± 0 <sup>aB</sup>	0 ± 0 <sup>aB</sup>	0 ± 0 <sup>aB</sup>	47*
	4	13.3 ± 1.6 <sup>bD</sup>	100 ± 0 <sup>eA</sup>	13.3 ± 1.6 <sup>bD</sup>	18.3 ± 3.3 <sup>bC</sup>	23.3 ± 3.3 <sup>bB</sup>	39*
	Malag.	100 ± 0 <sup>c</sup>	100 ± 0 <sup>f</sup>	100 ± 0 <sup>c</sup>	100 ± 0 <sup>c</sup>	100 ± 0 <sup>c</sup>	—
F		64*	180.5*	64*	30.2*	49*	
3	0 (ctrl)	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	—
	0.5	0 ± 0 <sup>aD</sup>	33.3 ± 4.4 <sup>bA</sup>	0 ± 0 <sup>aD</sup>	10 ± 0 <sup>bB</sup>	5 ± 0 <sup>aC</sup>	55*
	1	0 ± 0 <sup>aD</sup>	80 ± 5 <sup>cA</sup>	13.3 ± 1.6 <sup>bC</sup>	23.3 ± 3.3 <sup>cB</sup>	10 ± 0 <sup>aC</sup>	83*
	2	6.7 ± 1.6 <sup>bE</sup>	100 ± 0 <sup>dA</sup>	38.3 ± 3.3 <sup>cC</sup>	56.7 ± 4.4 <sup>dB</sup>	25 ± 2.8 <sup>bD</sup>	73*
	4	16.7 ± 1.6 <sup>cE</sup>	100 ± 0 <sup>dA</sup>	61.7 ± 4.4 <sup>dC</sup>	83.3 ± 3.3 <sup>eB</sup>	56.7 ± 4.4 <sup>cD</sup>	39*
	Malag.	100 ± 0 <sup>d</sup>	100 ± 0 <sup>d</sup>	100 ± 0 <sup>e</sup>	100 ± 0 <sup>f</sup>	100 ± 0 <sup>d</sup>	—
F		44.7*	89*	89.6*	105.2*	78*	
7	0 (ctrl)	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	—
	0.5	0 ± 0 <sup>aD</sup>	80 ± 2.8 <sup>bA</sup>	18.3 ± 3.3 <sup>bC</sup>	26.7 ± 1.6 <sup>bB</sup>	25 ± 2.8 <sup>bB</sup>	50*
	1	6.7 ± 1.6 <sup>aD</sup>	100 ± 0 <sup>cA</sup>	38.3 ± 3.3 <sup>cC</sup>	40 ± 2.8 <sup>cC</sup>	51.7 ± 1.6 <sup>cB</sup>	75*
	2	23.3 ± 3.3 <sup>bD</sup>	100 ± 0 <sup>cA</sup>	75 ± 5.7 <sup>dB</sup>	76.7 ± 4.4 <sup>dB</sup>	48.3 ± 6 <sup>cC</sup>	101*
	4	68.3 ± 3.3 <sup>cC</sup>	100 ± 0 <sup>cA</sup>	100 ± 0 <sup>eA</sup>	100 ± 0 <sup>eA</sup>	80 ± 2.8 <sup>dB</sup>	93*
	Malag.	100 ± 0 <sup>d</sup>	100 ± 0 <sup>c</sup>	100 ± 0 <sup>e</sup>	100 ± 0 <sup>e</sup>	100 ± 0 <sup>e</sup>	—
F		151.5*	48*	96.3*	147.7*	36.5*	

Means within a column, comparing the different concentrations of a fraction (small letters) and within a line, comparing the different fractions at a single concentration (capital letters) followed by the same letter do not differ significantly at  $P = 0.05$  (Student–Newman–Keuls's test).

\* $P < 0.01$ ; all cases  $df = 3, 15$ .



**Fig. 2.** Probit analysis of different solvent leaf fractions of *C. rigidus* in the laboratory at 1, 3, and 7 d after treatment against adult *C. maculatus*.

treated with hexane extract of cashew kernels when compared with methanol, ethanol, acetone, and pet-ether after 96 h of exposure. In addition, hexane and butanol fractions of *Ricinodendron heudelotii* (Baill) Pierre ex Pax (Euphorbiaceae) were more toxic than ethyl acetate, chloroform, and water fractions against adult *C. maculatus* 24 h posttreatment (Udo and Epidi 2009). Moreover, hexane extract of *Morinda lucida* (Benth.) (Rubiaceae) has been found more effective than acetone and methanol extracts against adult *C. maculatus* (Olufunmilayo 2012). Besides, hexane fraction of *D. arborea* showed more toxic activity than chloroform, ethyl acetate, butanol, and water fractions on adult *C. maculatus* 96 h postexposure (Epidi and Udo 2009). In a previous study, the following nine chemical classes: alkaloids, terpenoids, phenolic compounds (tannins), steroids, saponins,

and fixed oils were present in the leaves of *P. glandulosus* and those of *C. rigidus*. All the aforementioned nine chemical classes were found in the methanol crude extracts of the two plant species. For the two plant species, the chemical classes were similar for hexane (terpenoids, steroids, and fixed oils), ethyl acetate (tannins), and methanol (alkaloids, tannins, and saponins) fractions. Chloroform fraction of *P. glandulosus* contained only steroids and that of *C. rigidus*, terpenoids (Danga et al. 2014a, c). It has been demonstrated that treatments with natural compounds such as essential oils may cause symptoms that indicate neurotoxic activity including hyperactivity, seizures, and tremors followed by paralysis (knock down), which are very similar to those produced by the insecticides pyrethroids (Kostyukovsky et al. 2002). Steroids, known as modified triterpenoids, are biologically important natural products (Abe 2007). Terpenoids exhibit considerable toxicity to insects (Polosky et al. 1979). The combination of those actions may explain the very significant toxic effect of both plants shown in hexane fraction. Though the exact active molecules responsible of the toxic and progeny inhibition effects of both plants in this study are not yet known, plant-based extracts, i.e., azadirachtin have been commercialized and used for protecting stored products. NeemAzal powder showed very significant activity against *Sitophilus zeamains* in maize (Nukenine et al. 2011b). In addition, Calneem oil was found very effective against the tropical warehouse moth *Ephesia cautella* (Walker) (Lepidoptera: Pyralidae) in stored maize (Shehu et al. 2010). Two azadirachtin formulations exhibited very strong activities against adults of *Sitophilus oryzae* and *Tribolium confusum* on different grain commodities (Kavallieratos et al. 2007). Moreover, Calneem oil suppressed adult *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) on maize within 3 d of exposure (Adarkwah et al. 2010).

Alkaloids are a neuro-poison and powerful sterility agents for stored product pests (Jebanesan 2013). Recently, increasing insecticidal and repellent properties have been attributed to certain tannins (Amelio 1999). The pesticidal activity of saponins has long been reported. Saponin glycosides are very toxic to cold-blooded organisms (Jebanesan 2013). The different effects observed in each secondary metabolite may be responsible of moderate toxicity presented by one fraction or the other.

The different solvent leaf extracts and fractions of *P. glandulosus* and *C. rigidus* showed significant effect in reducing the number of

**Table 3. F<sub>1</sub> progeny production of adult *C. maculatus* in white bean grains treated with different solvent extract fractions of *P. glandulosus* and *C. rigidus* in the laboratory**

Crud. extr. and fractions	Dose (g/kg)	Mean number of F <sub>1</sub> adult progeny ± SE		Percentage of reduction in adult emergence relative to ctrl ± SE	
		<i>P. glandulosus</i>	<i>C. rigidus</i>	<i>P. glandulosus</i>	<i>C. rigidus</i>
Methanol crude extract	0	57.33 ± 2.03 <sup>a</sup>	61.33 ± 4.67 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>
	0.5	9.67 ± 2.03 <sup>b</sup>	12.67 ± 1.4 <sup>ab</sup>	83.23 ± 3.38 <sup>b</sup>	79.46 ± 1.01 <sup>b</sup>
	1	3 ± 0.58 <sup>c</sup>	6.33 ± 0.67 <sup>b</sup>	94.71 ± 1.09 <sup>c</sup>	89.41 ± 1.76 <sup>c</sup>
	2	1 ± 0 <sup>c</sup>	0 ± 0 <sup>c</sup>	98.25 ± 0.06 <sup>c</sup>	100 ± 0 <sup>d</sup>
	4	0 ± 0 <sup>c</sup>	0 ± 0 <sup>c</sup>	100 ± 0 <sup>c</sup>	100 ± 0 <sup>d</sup>
	Malagr.	0 ± 0 <sup>c</sup>	0 ± 0 <sup>c</sup>	100 ± 0 <sup>c</sup>	100 ± 0 <sup>d</sup>
	F	348.07*	137.24*	717.29*	142.63*
Hexane fraction	0	58.67 ± 2.60 <sup>a</sup>	63.33 ± 5.24 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0
	0.5	9.67 ± 0.67 <sup>b</sup>	0 ± 0 <sup>b</sup>	83.36 ± 1.89 <sup>b</sup>	100 ± 0
	1	2.33 ± 0.33 <sup>c</sup>	0 ± 0 <sup>b</sup>	95.96 ± 0.76 <sup>c</sup>	100 ± 0
	2	0 ± 0 <sup>c</sup>	0 ± 0 <sup>b</sup>	100 ± 0 <sup>d</sup>	100 ± 0
	4	0 ± 0 <sup>c</sup>	0 ± 0 <sup>b</sup>	100 ± 0 <sup>d</sup>	100 ± 0
	Malagr.	0 ± 0 <sup>c</sup>	0 ± 0 <sup>c</sup>	100 ± 0 <sup>d</sup>	100 ± 0
	F	433.28*	146.15*	226.87*	—
Chloroform fraction	0	49 ± 1.15 <sup>a</sup>	59.33 ± 3.76 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>
	0.5	6.33 ± 0.88 <sup>b</sup>	9 ± 1.15 <sup>ab</sup>	89.11 ± 0.72 <sup>b</sup>	84.84 ± 1.74 <sup>b</sup>
	1	1 ± 0 <sup>c</sup>	5.33 ± 0.33 <sup>b</sup>	97.96 ± 0.05 <sup>c</sup>	91 ± 0.28 <sup>c</sup>
	2	0 ± 0 <sup>c</sup>	0 ± 0 <sup>c</sup>	100 ± 0 <sup>d</sup>	100 ± 0 <sup>d</sup>
	4	0 ± 0 <sup>c</sup>	0 ± 0 <sup>c</sup>	100 ± 0 <sup>d</sup>	100 ± 0 <sup>d</sup>
	Malagr.	0 ± 0 <sup>c</sup>	0 ± 0 <sup>c</sup>	100 ± 0 <sup>d</sup>	100 ± 0 <sup>d</sup>
	F	1,070.18*	204.47*	393.08*	899.26*
Ethyl acetate fraction	0	38 ± 1.53 <sup>a</sup>	57.33 ± 8.09 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>
	0.5	9.67 ± 0.88 <sup>b</sup>	7.33 ± 0.67 <sup>b</sup>	74.47 ± 2.53 <sup>b</sup>	86.39 ± 2.93 <sup>b</sup>
	1	4.67 ± 0.67 <sup>c</sup>	1.67 ± 0.88 <sup>b</sup>	87.53 ± 2.34 <sup>c</sup>	100 ± 0 <sup>c</sup>
	2	2 ± 0 <sup>d</sup>	0 ± 0 <sup>b</sup>	95.58 ± 0.95 <sup>d</sup>	100 ± 0 <sup>c</sup>
	4	0 ± 0 <sup>d</sup>	0 ± 0 <sup>b</sup>	100 ± 0 <sup>d</sup>	100 ± 0 <sup>c</sup>
	Malagr.	0 ± 0 <sup>d</sup>	0 ± 0 <sup>b</sup>	100 ± 0 <sup>d</sup>	100 ± 0 <sup>c</sup>
	F	342*	46.19*	663.06*	106.09*
Methanol fraction	0	49.67 ± 1.76 <sup>a</sup>	56 ± 2.52 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>
	0.5	11.33 ± 0.88 <sup>b</sup>	11 ± 1 <sup>ab</sup>	77.01 ± 2.55 <sup>b</sup>	80.22 ± 2.26 <sup>b</sup>
	1	6.67 ± 0.33 <sup>c</sup>	7.67 ± 1.2 <sup>abc</sup>	86.94 ± 1.54 <sup>c</sup>	86.14 ± 2.53 <sup>c</sup>
	2	2.33 ± 0.33 <sup>d</sup>	4.67 ± 0.88 <sup>c</sup>	95.33 ± 0.5 <sup>d</sup>	91.64 ± 1.66 <sup>d</sup>
	4	0 ± 0 <sup>d</sup>	0 ± 0 <sup>d</sup>	100 ± 0 <sup>e</sup>	100 ± 0 <sup>e</sup>
	Malagr.	0 ± 0 <sup>d</sup>	0 ± 0 <sup>d</sup>	100 ± 0 <sup>e</sup>	100 ± 0 <sup>e</sup>
	F	506.28*	271.94*	925.32*	579.74*

Means within a product followed by the same letter do not differ significantly at  $P = 0.05$  (Student–Newman–Keuls's test).

\* $P < 0.01$ ; all cases  $df = 3, 15$ .

F<sub>1</sub> progeny. The activity varied from a fraction to the other with hexane fraction of *C. rigidus* being the most active to register no progeny production at all doses. Some researchers also got similar results of *C. maculatus* progeny inhibition in cowpea treated with plant extracts and fractions. Hexane, chloroform, ethyl acetate, butanol, and water fractions of *R. heudelotii* showed significant effect in reducing the number of F<sub>1</sub> progeny of *C. maculatus* (Udo and Epiidi 2009). The same solvent fractions of *D. arborea* greatly inhibited F<sub>1</sub> progeny of *C. maculatus* (Epiidi and Udo 2009). The reduction of adult emergence could be explained in part by the antiovipositional, ovicidal, and reproductive properties of phytochemicals present within both plant fractions.

In this study, the tested concentrations (0.5, 1, 2, and 4 g/kg) were higher in comparison with that of conventional Malagrain (0.5 g/kg). To overcome this situation, it may be suggested to fractionate the crude extracts by combining the solvents in different ratios. Synergist compounds within mixtures may lead to the development of more effective insecticides as well as the use of smaller amounts in the mixture to achieve satisfactory levels of efficacy.

The results obtained in this study suggest that hexane fraction of *P. glandulosus* and *C. rigidus* leaves can be followed up for further fractionation. In light of our findings, a project is in view for the bioassay-guided fractionation of hexane fraction from both plants to elucidate the molecule(s) responsible of the toxicity to *C. maculatus*. However, more research dealing with potential effects of the fractions on the organoleptic characteristics of the commodity have to be carried out before promoting the use of these fractions.

## Acknowledgments

We gratefully acknowledge the equipment facilities provided by the Faculty of Pharmaceutical Sciences, Agulu, Anambra state, Nigeria, and Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. We also thank the staff members of the same institution for their cooperation. We are extremely indebted to the anonymous reviewers who made wonderful comments and suggestions, which greatly improved the quality of this article.

## References Cited

- Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18: 265–267.
- Abdel-Mogib, M., H. A. Albar, and S. M. Batterjee. 2002. Chemistry of the genus *Plectranthus*. *Molecules* 7: 271–301.
- Abe, I. 2007. Enzymatic synthesis of cyclic triterpenes. *Nat. Prod. Rep.* 24: 1311–1331.
- Adarkwah, C., D. Obeng-Ofori, C. Büttner, C. H. Reichmuth, and M. Schöller. 2010. Bio-rational control of red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) in stored wheat with Calneem oil derived from neem seeds. *J. Pest Sci.* 83: 471–479.
- Adedire, C. O., and J. O. Akinneye. 2003. Biological activity of the Marigold, *Tithonia diversifolia*, on cowpea seed bruchid, *Callosobruchus maculatus* (F) (Coleoptera: Bruchidae). *Ann. Appl. Biol.* 144: 185–189.
- Adedire, C. O., O. M. Obembe, R. O. Akinkulore, and S. O. Oduleye. 2011. Response of *Callosobruchus maculatus* (Coleoptera: Chrysomelidae: Bruchinae) to extracts of cashew kernels. *J. Plant Dis. Protect.* 118: 75–79.
- Ali, S. M., S. M. Mahgoub, M. S. Hamed, and M. S. A. Gharib. 2004. Infestation potential of *Callosobruchus chinensis* and *Callosobruchus*

- maculatus* on certain broad bean seed varieties. Egypt. J. Agric. Res. 82: 1127–1135.
- Amelio, S. D. 1999.** Constituents of drugs. Botanicals, pp. 1–6. CFC Press, Boca Raton, London.
- Csurhes, S. 2008.** Pest plant risk assessment: neem tree, *Azadirachta indica*, 16 p., Queensland the Smart State.
- Danga, Y. S. P., C. O. Esimone, and E. N. Nukenine. 2014a.** Larvicidal and phytochemical properties of *Callistemon rigidus* R. Br. (Myrtaceae) leaf solvent extracts against three vector mosquitoes. J. Vector Borne Dis. 51: 216–223.
- Danga, Y. S. P., C. O. Esimone, L. Younoussa, and E. N. Nukenine. 2014b.** Larvicidal and pupicidal activities of *Plectranthus glandulosus* and *Callistemon rigidus* leaf essential oils against three mosquito species. J. Mosq. Res. 4: 5–14.
- Danga, Y. S. P., E. N. Nukenine, L. Younoussa, and C. O. Esimone. 2014c.** Phytochemicals and larvicidal activity of *Plectranthus glandulosus* (Lamiaceae) leaf extracts against *Anopheles gambiae*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). J. Pure Appl. Zool. 2: 160–171.
- Devine, G. J., and M. J. Furlong. 2007.** Insecticide use: contexts and ecological consequences. Agric. Hum. Val. 24: 281–306.
- Diouf, D. 2011.** Recent advances in cowpea (*Vigna unguiculata* (L.) Walp) “omics” research for genetic improvement. Afr. J. Biotechnol. 10: 2803–2810.
- Epidi, T. T., and I. O. Udo. 2009.** Biological activity of ethanolic extract fractions of *Dracaena arborea* against infestation of stored grains by two storage insect pests. Pakistan J. Biol. Sci. 12: 976–980.
- Finney, D. J. 1971.** Probit analysis, 3rd ed., p. 68–72. Cambridge University Press, Cambridge.
- Hong, T., S. Qi, Z. Xiaomao, and B. Lianyang. 2013.** Field resistance of *Spodoptera litura* (Lepidoptera: Noctuidae) to organophosphates, pyrethroids, carbamates and four newer chemistry insecticides in Hunan, China. J. Pest Sci. 86: 599–609.
- Isman, M. B. 1999.** Pesticides based on plant essential oils. Pestic. Outlook 10: 68–72.
- Isman, M. B. 2006.** Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu. Rev. Entomol. 51: 45–66.
- Jebanesan, A. 2013.** Secondary metabolites of plants used in pest control. Int. J. Curr. Mol. Res. 1: 12–15.
- Kamanula, J., G. W. Sileshi, S. R. Belmain, P. Sola, B. M. Mvumi, G. K. C. Nyirenda, S. P. Nyirenda, and P. C. Stevenson. 2010.** Farmers’ insect pest management practices and pesticidal plant use in the protection of stored maize and beans in Southern Africa. Int. J. Pest Manage. 57: 41–49.
- Kavallieratos, N. G., C. G. Athanassiou, C. J. Saitanis, D. C. Kontodimas, A. N. Roussos, M. S. Tsoutsas, and U. A. Anastassopoulou. 2007.** Effect of two azadirachtin formulations against adults of *Sitophilus oryzae* and *Tribolium confusum* on different grain commodities. J. Food Protect. 70: 1627–1632.
- Kostyukovsky, M., A. Rafaeli, C. Gileadi, N. Demchenko, and E. Shaaya. 2002.** Activation of octopaminergic receptors by essential oil constituent isolated from aromatic plants: possible mode of action against insect pest. Pest Manage. Sci. 58: 1101–1106.
- Magadula, J. J., E. Innocent, and J. N. Otieno. 2009.** Mosquito larvicidal and cytotoxic activities of 3 *Annona* species and isolation of active principles. J. Med. Plant Res. 3: 674–680.
- Ngamo, T.S.L., H. Kouninki, Y. D. Ladang, M. B. Ngassoum, P. M. Mapongmestsem, and T. Hance. 2007a.** Potential of *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) as biocontrol agent of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). Afr. J. Agric. Res. 2: 168–172.
- Ngamo, T.S.L., M. B. Ngassoum, P. M. Mapongmestsem, and W. F. Noudjou. 2007b.** Use of essential oils of aromatic plants as protectant of grains during storage. Agric. J. 2: 204–209.
- Ntonifor, N. N., O. E. Oben, and B. C. Konje. 2010.** Use of selected plant-derived powders and their combinations to protect stored cowpea grains against damage by *Callosobruchus maculatus*. J. Agric. Biol. Sci. 5: 13–21.
- Nukenine, E. N., C. Adler, and C. Reichmuth. 2007.** Efficacy evaluation of plant powders from Cameroon as post-harvest grain protectants against the infestation of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). J. Plant Dis. Protect. 114: 30–36.
- Nukenine, E. N., J. W. Goudougou, C. Adler, and C. Reichmuth. 2010.** Efficacy of diatomaceous earth and botanical powders against the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) on maize, Vol. 425, pp. 881–887. Tenth International Working Conference on Stored Product Protection, Julius-Kühn-Archiv.
- Nukenine, E. N., C. Tchiegang, A. A. T. Mekouo, K. H. Tofel, C. Adarkwah, D. Obeng-Ofori, and C. Adler. 2011a.** Efficacy of Calneem derived from Ghanaian neem seeds and seed oils from two locations in Cameroon against *Sitophilus zeamais* (Coleoptera: Curculionidae) on maize. Int. J. Trop. Insect Sci. 31: 225–234.
- Nukenine, E. N., H. K. Tofel, and C. Adler. 2011b.** Comparative efficacy of NeemAzal and local botanicals derived from *Azadirachta indica* and *Plectranthus glandulosus* against *Sitophilus zeamais* on maize. J. Pest Sci. 84: 479–486.
- Nukenine, E. N., F. P. Chouka, M. B. Vabi, and C. Reichmuth, C. Adler. 2013.** Comparative toxicity of four local botanical powders to *Sitophilus zeamais* and influence of drying regime and particle size on insecticidal efficacy. Int. J. Biol. Chem. Sci. 7: 1313–1325.
- Oliver-Bever, B. 1982.** Medicinal plants in West Africa. Cambridge University Press.
- Ojo, J. A., A. A. Olunloyo, and E. O. Akanni. 2013.** Efficacy of *Moringa oleifera* leaf powder against *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) on stored cowpea (*Vigna unguiculata* L. Walp). Res. 5: 240–244.
- Okoye, F. B. C., and O. P. Osadede. 2009.** Study on the mechanism of anti-inflammatory activity of the extracts and fractions of *Alchornea floribunda* leaf. Asian Pac. J. Trop. Med. 2: 7–14.
- Olufunmilayo, E. A. 2012.** Bioactivity of the leaf extracts of *Morinda Lucida* (Benth.) against cowpea bruchid, *Callosobruchus Maculatus* (F.) (Coleoptera: Chrysomelidae). Exp. Agric. Hortic. 1: 1–7.
- Oluwafemi, D. A., L. O. Adebayo, B. Kehinde, and O. Omoche. 2013.** Evaluation of four cowpea lines for bruchid *Callosobruchus maculatus* tolerance. J. Nat. Sci. Res. 3: 46–51.
- Pangnakorn, U. 2009.** Efficiency of Vetiver Grass extracts against Cowpea Weevil (*Callosobruchus maculatus* Fabr.). Am Eurasian J. Agric. Environ. Sci. 6: 356–359.
- Polosky, J., Z. Varon, B. Arnoux, C. Pascard, G. R. Petit, J. M. Schmidt, M. Ochi, and H. Kotsuki. 1979.** Cytotoxic effect of terpenoids from *Aphanomixis grandiflora*. Phytochemistry 30: 1198–1201.
- Shehu, A., D. Obeng-Ofori, and V. Y. Eziah. 2010.** Biological efficacy of calneem oil against the tropical warehouse moth *Ephesia cautella* (Lepidoptera: Pyralidae) in stored maize. Int. J. Trop. Insect Sci. 30: 207–213.
- Stevenson, P. C., G. C. Kite, G. P. Lewis, F. Forest, S. P. Nyirenda, S. R. Belmain, G. W. Sileshi, and N. C. Veitch. 2012.** Distinct chemotypes of *Tephrosia vogelii* and implications for their use in pest control and soil enrichment. Phytochemistry 78: 135–146.
- Suleiman, M., N. D. Ibrahim, and Q. Majeed. 2012.** Control of *Sitophilus zeamais* (Motsch) (Coleoptera: Curculionidae) on Sorghum using some plant powders. Int. J. Agric. For. 2: 53–57.
- Tofel, H. K., E. N. Nukenine, D. Ulrich, and C. Adler. 2014.** Effect of drying regime on the chemical constituents of *Plectranthus glandulosus* leaf powder and its efficacy against *Callosobruchus maculatus* and *Sitophilus zeamais*. Int. J. Agron. Agric. Res. 5: 80–91.
- Udo, I. O., and T. T. Epidi. 2009.** Biological effect of ethanolic extract fractions of *Ricinodendron heudelotii* (Baill) Pierre ex Pax against *Sitophilus zeamais* Motschulsky and *Callosobruchus maculatus* Fabricius on stored grains. Afr. J. Agric. Res. 4: 1080–1085.
- Umeozor, O. C. 2005.** Effect of the infection of *Callosobruchus maculatus* (F.) on the weight loss of stored cowpea (*Vigna unguiculata* (L.) Walp). J. Appl. Sci. Environ. Manage. 9: 169–172.
- Wiesbrook, M. L. 2004.** Natural indeed: are natural insecticides safer and better than conventional insecticides? Ill. Pestic. Rev. 17: 8.

Received 22 April 2015; accepted 1 September 2015.