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Toxicity of powdered and ethanolic extracts of *Uvaria chamae* (*Annonaceae*) Bark on selected stored product insect pests

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A study was carried out on the insecticidal effects of the powdered stem bark extract of *Uvaria chamae* and its ethanolic extract on three most devastating stored products pests (Coleopterous) in Nigeria, namely: *Callosobruchus maculatus* F. (Bruchidae), *Rhizopertha dominica* F. (Bostrichidae) and *Sitophilus zeamais* Motschulsky (Curculionidae). Graded concentrations of each formulation of the powdered bark and ethanolic extracts were in exposure chambers of each insect in laboratory bio-assays under ambient conditions (25±2°C). *S. zeamais, R. dominica* and *C. maculatus* were exposed to the following concentrations 0.00, 0.10, 0.20, 0.40, 0.80, and 1.60 mg/L grains in three replicates per treatment and control. The mortality of the insects was used to compute mean lethal concentration (LC_{50}) values by probit analysis. All the concentrations tested showed appreciable toxicity against each test insect species. The computed LC_{50} values for powder formulation gave significantly ($P \leq 0.05$) higher toxicity against *C. maculatus* (1.281 g/kg) than either *S. zeamais* (2.145 g/kg) or *R. dominica* (5.189 g/kg). However, the ethanolic extract was more toxic on *C. maculatus* (0.134 mL/L), *S. zeamais* (0.173 mL/L) or *R. dominica* (0.359 mL/L). It was found that the higher the concentration of the ethanolic extract, the higher the mortality. The result implies that, *U. chamae* powdered and ethanolic stem bark extracts have potentials for use during storage of grains, ensuring food security, profit maximization and availability of seeds for the next planting season without being damaged by these test insect species. The presence of high concentration of steroids and terpenes may be responsible for the observed high insecticidal activity of the test extracts.

Key words: Bioassay, *Callosobrochus maculatus*, storage insect, *Uvaria chamae*.

INTRODUCTION

The storage of crops is a deliberate policy in most countries to guarantee the populace freedom from hunger, malnutrition and deprivation through actions that ensures adequate and consistent food supply at affordable prices. According to Ihimodu (2004), the food self-sufficiency ratio has fallen from 98% in the early 1960s to less than 54% in 1986. In 1990, 18% of the Nigerian population (14.4 million) was estimated to be critically food insecure and this has increased to 36% (32.7 million) in 1992 and further increased to 40.7% in

1996 (Babatunde and Oyatoye, 2005). Presently, over 40% of Nigeria's estimated population of 133 million people is food insecure (Idachaba, 2004). Food security in sub-Saharan Africa largely depends on improved food productivity through the use of sustainable Good Agricultural Practices (GAPs) and the reduction of postharvest losses caused by pests and diseases (Babatunde and Oyatoye, 2005).

Insects form more than 75% of the population of known animals and constitute the major factor limiting agricultural food production. About 10.84 million metric tons of cereals and almost a million tons of legumes are produced annually and an average of between 1.5 and 2 million tons are lost to heavy insect pest infestations and mould within poor storage system (FAO, 2011). Losses of about six million tons of grains per annum are incurred by insects both in the field and stores translating to five billion naira per annum (Bogunjoko, 1987). Ahmed (2013) reported that post-harvest losses is making Nigerian farmers poorer and Patrick (2013) reported that Nigeria records over 40% post-harvest losses, which has led to an unprecedented hike in food importation in the country. According to FAO (2011), about one third of food for human consumption is lost or wasted globally to about 1.3 billion tons per year. Also, 30 to 40% of the food crops produced worldwide is never consumed as a result of damage, rotting as well as pest and diseases which affect the crops after harvest (Meena et al., 2009).

Nigeria is the largest producer and consumer of cowpea in the world (Lowenberg-Deboer and Ibro, 2008; Pereira et al., 2001) and it was estimated by FAO that 3.3 million tons of cowpea dry grains were produced in 2000 (IITA, 2001), but only a small proportion enters international trade due to losses by insects pests during storage. Similarly, maize is also one of the most important cereal crops grown from the coast to the savannah (IITA, 2009); however, weight losses of 10 to 30% have been recorded in maize stored for 3 to 6 months (Samuel et al., 2011) due to insect pests. Although synthetic insecticides are effective and quick in action, they are not eco-friendly and are mostly toxic if consumed. Safer and more environment-friendly alternative methods of controlling insect pests on stored grains are therefore needed. Some studies have shown that botanicals may serve as such alternatives (Denloye et al., 2007). Botanical insecticides remain important in insect pest management because they are believed to provide the most effective control against insect pests that have become resistant to other insecticides (Weinzierl, 2000). They may provide sustainable, safe, available and cheap alternative to synthetic insecticides in the control of storage insect pests threatening stored food and these have led to the belief that plant-derived insecticides are safer and more ecofriendly than synthetic products. However, there is little information on the use of *Uvaria chamae* bark as biopesticide. This study was therefore aimed at investigating the insecticidal efficacy of the powdered and ethanolic stem bark extracts of *U. chamae* against *Callosobruchus maculatus* F. (Bruchidae), *Rhizopertha dominica* F. (Bostrichidae) and *Sitophilus zeamais* Motschulsky (Curculionidae).

MATERIALS AND METHODS

Plant

Fresh bark of *U. chamae* was procured from vendors at Oyingbo market in Lagos State and identified at the herbarium of Botany Department, University of Lagos, Nigeria. The test plant bark was dried to constant weight in the oven at 50°C for 8 days, and then pulverized by first pounding in a mortar before using a micro-hammer mill to grind into powder. The powder was passed through a sieve of 0.1 mm mesh size in order to standardize particle size.

Preparation ethanol extracts

Test plant powder (500 g) was wrapped in a clean dry muslin cloth and then placed inside the thimble of a Soxhlet apparatus and 1 L of 80% ethanol then poured into a round bottom flask. The apparatus was heated at 60°C using a heating mantle. The experiment was left to run through several refluxes for 6 h until a colourless liquid was observed in the capillary tube. The resultant filtrate was then concentrated over a water bath at 40°C, kept in specimen bottle and refrigerated until needed for bioassay. For the ethanol extraction, 0.5 and 10 mg/L concentrations of the various test plant materials were used, while the controls were carried out exactly the same way except that the grains were treated with ethanol.

Disinfestation of cowpea and maize seeds

Cowpea seeds (*Vigna unguiculata* [L.] Walp.var. Tvu 3629) and maize grains (*Zea mays*. var. TZESR-20) were obtained at the Bariga market, Lagos. They were identified at the International Institute of Tropical Agriculture (IITA), Ibadan. All damaged seeds and debris were sorted out from the grains after which disinfestation was carried out in an oven at 50°C for 6 h to kill all life stages of insects within the grains. The grains were then left for 24 h to stabilize at ambient conditions.

Culture of test insects

C. maculatus was maintained on the disinfested cowpea seeds. 500 g of cowpea seeds in five replicates were weighed into clean 1 L Kilner jars. Fifty 0 to 3 day old unsexed adults were introduced into the jar and covered with muslin cloth held in place by rubber band. *S. zeamais, R. dominica* and *C. maculatus* were exposed to the following concentrations 0.00, 0.10, 0.20, 0.40, 0.80, and 1.60 mg/L grains in three replicates per treatment and control. After 5 days, the insects were removed and left undisturbed until insect

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Table 1. Toxicity of *Uvaria chamae* seed powder and Actellic dust against test insects (df=3).

Treatment	Test insects	48 h LD_{50} (g/kg)	95% Confidence limits	Regression equation (Y)	Slopes (Standard error)
U. chamae powdered seed	*Callosobruchus*	1.281	4.424-3.429	-0.823+0.96x	0.955±0.310
	Sitophilus	2.145	0.894-5.826	-0.286+0.865x	0.865±0.307
	Rhizopertha	5.189	3.258-9.880	1.614+0.38x	-1.1541±0.614
Actellic dust	*Callosobruchus*	0.048	0.010-0.127	1.816+1.380x	1.380±0.330
	Sitophilus	0.142	0.001-0.388	0.935+0.105x	1.105±0.390
	Rhizopertha	0.201	0.016-0.401	-1.204+0.727x	1.727±0.532

emergence was observed. At each peak of emergence, the insects were sieved out and new cultures were set up to ensure regular supply of adult insects of known age for experimentation. Similarly, *S. zeamais* and *R. dominica* were separated and maintained on disinfested maize grains. Fifty unsexed 7 to 14 day old adults of *S. zeamais* were introduced into 500 g of the disinfested maize grains in 1 L Kilner jars in five replicates in the laboratory. All adult insects were left for 7 days to allow for oviposition, after which they were removed. They were then left undisturbed until adults emerged. At each peak of emergence, the adults were removed and used to set up new cultures. Series of fresh cultures were made from these ones to ensure regular supply of adult insects of known age for use in subsequent experiments. Similar method was used for preparation of *R. dominica* cultures.

Laboratory bioassays

Powdered and ethanolic extracts of the bark of *U. chamae* were respectively screened against each experimental insect species following the method of Denloye et al. (2007). For these series of experiments, the prepared plant powder and extracts were respectively tested against each test insect species in elaborate bioassays to measure acute toxicity levels dependent on 48 h LC_{50} values. For the test plant powder, 20 unsexed 2 to 3 day old adult insects were exposed per replicate of each treatment and control. The insects were treated to admixture of plant powder and cowpea (for *C. maculatus*) or maize (for *S. zeamais* or *R. dominica*) at concentration of 0.125 to 8.00 g/kg grain. For the ethanolic extract, the grains were dipped in extracts of 0.10 to 1.60 g/L concentrations. Dipping was carried out by completely immersing seeds in each extract concentration for 30 s. All dipped grains were allowed to drain on filter paper for 5 min before transferring them into bioassay containers. Several sets of 40 cowpea seeds were at these concentrations with untreated seeds used as control. All experiments and control were set up at the same time. Each treatment and control was replicated four times. In all treatments and control, mortality of each of the exposed test insect species was assessed every 24 h for 2 days.

Quantitative determination of chemical constituents of test plant materials

This was carried out on the plant extracts obtained following standard procedures of the Association of Official Agricultural Chemists (AOAC, 2000) for alkaloids, flavonoids, tannins, cardiac glycosides, cyanogenic glycosides, anthraquinone, glycosides, saponins, anthocyanides, anthocyanin pigments, reducing sugar compounds and phlobatannins.

Gas chromatography-mass spectrometry (GC-MS)

The test ethanolic extract of *U. chamae* (5 g) was soaked in 50 mL hexane for 2 days, and extracted again with 20 mL hexane three times concurrently. The extract was then concentrated in water bath. GC-MS analysis was performed using an Agilent 5975C gas chromatograph apparatus equipped with an Agilent mass spectrometric detector, a direct capillary interface and fused silica capillary column HP-5MS (30 m × 0.32 mm, film thickness 0.25 µm). Helium was used as carrier gas at approximately 1.0 ml/min, pulsed split less mode. The solvent delay was 4 min and the injection size was 1.0 µL. This pushed the samples injected into the columns, being the stationary phase which has been conditioned to 320°C for 2 h for separation. The column was pumped down to allow for stability. The analyses were carried out in duplicate for each sample batch. The individual peaks were identified by comparison of their retention indices to those of available authentic samples using the Wiley and pesticides mass spectral database library.

Data analysis

The number of dead test insects at all treatments in acute toxicity experiments were corrected using Abbott's (Abbott, 1925) formula. Probit analyses were then carried out following Finney (1971) protocol and median lethal concentrations (LC_{50}) values were obtained based on a computer programme. Values with overlapping confidence limits were not significantly different.

RESULTS

Toxicity of *U. chamae* powder stem to the test insects relative to Actellic dust

The test plant powder and actellic dust, respectively were toxic to all test insect species, although actellic demonstrated a much higher insecticidal effect against each of the test insects than *U. chamae* powder (Table 1). The LC_{50} values computed for *C. maculatus* (1.28 g/kg), *S. zeamais* (2.15 g/kg) and *R. dominica* (5.19 g/kg) in Table 1 indicate that the test plant powder was more potent against *C. maculatus* than any of the other insects. There was however no significant difference in the toxicity of the powder to the test insects due to overlap in the 95% confidence limits. The toxicity factor of actellic dust when compared with the effects of *U. chamae* on the

Table 2. Toxicity of *Uvaria chamae* ethanolic extract against test insects (df=3).

Test insects	48 h LC_{50} (mg/L)	95% Confidence limits	Regression equations	Slopes (± Standard error)
Callosobruchus	0.134	0.025-0.245	Y= 0.939+1.076x	1.076±0.334
Sitophilus	0.173	0.049-0.301	Y= 0.840+1.103x	1.103±0.328
Rhizopertha	0.359	0.079-0.626	Y=0.617+1.387x	1.387±0.370

Table 3. Percentage mortality of *S. zeamais* adults treated with test plant ethanolic extracts.

Organism	Concentration	%Mortality
S. zeamais	0.1	22.0 (4.74%)[a]
	0.2	28.5 (4.89%)[a]
	0.4	41 (6.44%)[a]
	0.8	56.5 (7.55%)[ab]
	1.6	82.0 (9.08%)[c]
R. dominica	0.1	18.5 (4.56%)[b]
	0.2	26.5 (5.19%)[b]
	0.4	40 (6.36%)[b]
	0.8	51.5 (7.21%)[b]
	1.6	76.0 (8.75%)[c]
C. maculates	0.1	18.5 (4.36%)[a]
	0.2	30 (5.5%)[a]
	0.4	50 (7.11%)[a]
	0.8	61.0 (7.84%)[b]
	1.6	88 (9.41%)[c]

Each datum is a mean of three replicates. Values in parenthesis are square roots (√x + 0.5) transformed. Mean values bearing the same letters are not significantly different by LSD at P = 0.05.

test insects showed that it was 15.1, 25.8 and 26.7 times more toxic to *S. zeamais, R. dominica* and *C. maculatus,* respectively.

Toxicity of *U. chamae* ethanolic extract to the test insects

The test plant extract was more toxic to *C. maculatus* than either *S. zeamais* or *R. dominica* (Table 2). The LC_{50} values computed for *C. maculatus* (0.134 mg/L), *S. zeamais* (0.173 mg/L) and *R. dominica* (0.359 mg/L g/kg) in Table 1 indicate that the test plant powder was more potent against *C. maculatus* than any of the other insects. There was however no significant difference in the toxicity of the extract to the test insects due to overlap in the 95% confidence limits. The computed LC_{50} values shows that the *U. chamae* ethanolic extract was significantly more toxic to *C. maculatus* (0.134 mg/L) than *R. dominica* (0.359 mg/L) with confidence limits not overlapping (Table 2).

However, the result for the rate of mortality induced by various ethanolic concentrations of *U*. *chamae* to the organisms is shown in Table 3. The result indicated high percentage mortality among insects treated with higher concentrations of the ethanolic extracts of *U. chamae*. The effect increases with increase in concentration.

Chemical constituents in *U. chamae* ethanolic extract

The qualitative analysis indicated that *U. chamae* contained alkaloids, catechol tannins, condensing tannins, cardiac glycosides, flavonoids, reducing sugar, saponins, anthraquinone, phlobatannins, hexose's'-sugar, Keto-sugar Pento-sugar and monosaccharide (Table 3). The major constituents identified by Gas Chromatography/Mass Spectrometer analyses in *U. chamae* were benzene derivatives (52.9%), followed by aliphatic compounds dominated by higher acids, alcohols

Table 4. Quantitative determination of constituents present in *U. chamae* extracts.

Analysis	Constituents
Alkaloid analysis	
Dragendoff's reagent	+
Meyer's reagent	+
Test for Tannins	
Ferric chloride test	+
Ferric bromine water	+
Cardiac-Glycoside analysis	
Legal test.	+
Kedde test	-
Steroidal test	
Lieberman's test	+
Salkowski test	+
Flavonoid analysis	
Ferric chloride test	+
Lead acetate test	+
Saponins	
Benedict's test	+
B. Frothing test	+
Anthraquinones analysis Borntrager's test	
Phlobatannin's test	
Reducing sugar analysis	
Barfoed test	-
Resorcing test	+

and hydrocarbons (38.6% fatty acids), terpenoids with total percentage peak area of 7.39% and quinolines 1.09% (Table 4).

DISCUSSION

The biological activities of the dusts and ethanol extracts of the test plants were different in their efficacies, with the ethanolic extracts being the most effective followed by the dust formulation. This corroborates the reports of Obi and Onuoha (2000) and Ogueke et al. (2006) who independently reported that ethanol is the best solvent for the extraction of most plant active ingredients. The differences in toxicity between the ethanolic extract and dusts formulation agree with the findings of Benner (1993) who observed that the active materials in plant extracts are more concentrated than in the plant dusts, hence the reason for their higher potency. Lale and Mustapha (1999) also reported that extracts of neem were significantly more potent in reducing oviposition and adult emergence of *C. maculatus* than the powdered form.

The effect of dust on *C. maculatus* might be due to their action as physical barriers that deter free movement or access of ovipositing adults to suitable sites on the seeds or clog the insect spiracles and trachea causing suffocation or may be that the cuticle of the insect might have suffered abrasion which could result in dehydration therefore causing stress and death which has a direct relationship with particle size of dusts as stressed by Sousa et al. (2005). This could probably be the case with *C. maculates* with a thinner and less sclerotized cuticle than *S. zeamais* and *R. dominica,* making it more susceptible to the dust formulation than the other test insects.

However, mortality in S*. zeamais* and *R. dominica* might be due to feeding on treated grains as suggested by Wolfson et al. (1991) although this does not apply to *C. maculatus* adults since they do not feed except there is proof that the developing larvae fed on the treated plant materials. This could have only been the case if the extract penetrated into the seed, since the larvae feeds within the seeds. The toxicity of *U. chamae* against the adult test insects agrees with the work of Lajide et al. (1998) who reported that pulverized seeds of *Uvaria afzelli* (although a different species from the one used in this study) were found to be highly toxic to maize weevil when used as surface treatment of maize grains subsequently infested with the weevils.

The presence of saponins, terpenes and cardiac glycosides in *U. chamae* is similar to the findings of Oluremi et al. (2010), Okon et al. (2013), Kone et al. (2013) and Osoagwu and Ihenwosu (2014) as they all advocated that toxicity to various effects of terpenes in plants to insects as stressed by Adebowale and Adedire (2006). The presence of high proportion of steroids and terpene alcohols in *U. chamae* is probably responsible for its insecticidal activity.

Conclusions

The insecticidal activity of *U. chamae* extracts was found to be effective against *S. zeamais, R. dominica* and *C. maculatus.* It was found that both the ethanolic extracts and powdered extracts can be used to control these devastating pests. However, for easy usage, the use of powdered extract is recommended as it can easily be prepared and highly effective ecofriendly insecticide to the synthetic insecticides.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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