Toxicity of powder and extracts of *Zanthoxylum zanthoxyloides* Lam (Rutaceae) root bark from Nigeria to three storage beetles

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Abstract

The root of *Zanthoxylum zanthoxyloides* Lam is used as antibacterial toothbrush in southwestern Nigeria. The root bark was therefore screened as powder, aqueous and ethanolic extracts for toxicity to adult *Callosobruchus maculatus* F. (Coleoptera: Bruchidae), *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and the effects of the test extracts on oviposition and progeny development of *C. maculatus* in laboratory tests. A small scale field trial was also carried out to test the efficacy of test powder as a protectant of cowpea, *Vigna unguiculata* (L.) Walpers and maize, *Zea mays* L. grains against insect infestation. Results of the acute toxicity tests showed that all the formulations were toxic to the insects. The 48 h median lethal concentration (LC_{50}) values obtained for the test powder against *C. maculatus*, *S. zeamais* and *T. castaneum* are 0.05 g kg^{-1}, 0.01g kg^{-1} and 0.04 g kg^{-1}, respectively. For the aqueous extracts the LC_{50} values are 0.83 g L^{-1}, 0.34 g L^{-1} and 0.38 g L^{-1} against *C. maculatus*, *S. zeamais* and *T. castaneum*, respectively while the values are 0.02 g L^{-1}, 0.04 g L^{-1} and 0.09 g L^{-1}, respectively for ethanolic extract, indicating higher toxicity against the test insects relative to the water-based extract. The ethanolic extract demonstrated residual property, the toxicity to *C. maculatus* remaining fairly constant over a total post-treatment time of 336 h. Cowpea grain treatment with test plant ethanolic extract resulted in reduction of the number of eggs laid from 93.30 ± 3.46 in the control to 21.00 ± 4.57 in grain treated with 0.10 g L^{-1} extract without significant difference in the number of adult emergence from the treated grains. Field trials showed that cowpea and maize grains treated with test plant powder respectively were protected from insect infestation for 180 d. These results demonstrate the potentials of *Z. zanthoxyloides* for protecting cowpea and maize grains against storage insects.

Keywords: *Zanthoxylum zanthoxyloides, Callosobruchus maculatus, Sitophilus zeamais, Tribolium castaneum,* Toxicity

1. Introduction

Insects damage stored grains and also create conditions that allow secondary infestation by other pests and deterioration by microorganisms, primarily fungi (Agrawal et al., 1988; Oke and Muniru, 2001). Once an infestation is established insect pests generally cause gradual and progressive damage, leading to losses in nutritional, organoleptic and aesthetic quality as well as weight loss to stored grains. About 40 insect species can damage grains (Osuji, 1985; Sousa et al., 2005), including the cowpea weevil, *Callosobruchus maculatus* F. (Coleoptera: Bruchidae), the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) and the red flour beetle, *Tribolium castaneum* (Herbst) (Colepotera: Tenebrionidae). While *C. maculatus* and *S. zeamais* are primary pests attacking intact cowpea, *Vigna unguiculata* Walpers (Fabaceae) seeds and maize, *Zea mays* L. (Poaceae) grains, *T. castaneum* infests grains and other products including groundnut, *Arachis hypogea* L. (Fabaceae) during post-harvest handling. These insects are responsible for up to 80 % of the infestations in infestation on cowpea, maize and groundnut during storage (Osuji, 1985; Jood et al., 1996), thus justifying control measures to protect these crops.

It is well established that many synthetic insecticides are effective in controlling insects in stored products. However, some of these insecticides can have deleterious side effects and the costs of application are excessive for many developing countries. These limitations necessitates search for new insecticides with novel mechanisms of action. In this regard, it the bioactivity of botanicals, particularly edible plant species, have been investigated as sources of insecticides that are safer to use (Golob and

Webley, 1980; Don-Pedro, 1984; Don-Pedro, 1985), and more easily and cheaply produced as crude or partially purified extracts (Rahman and Talukder, 2006), which would benefit subsistence level storage in developing countries. Thus, we conducted studies using *Zanthoxylum zanthoxyloides* Lam (Rutaceae). The root of this plant is commonly used as toothbrush because it has antimicrobial effect.

Traditionally in Africa, the storage time for grains is between three and six months before they are consumed, processed into livestock feed or used as seeds for the next planting season. More importantly, these grains are seasonal. Long-term studies on the insecticidal effect of plant species in protecting and controlling existing infestation of stored grains under ambient conditions in the field is therefore necessary.

The purpose of the present study is to determine the toxicity of powder, aqueous extract and ethanolic extract of *Z. zanthoxyloides* on adult *C. maculatus, S. zeamais* and *T. castaneum,* investigate the effect of the ethanol extract on oviposition and adult emergence, and assess the ability of the plant materials to protect stored cowpea and maize grains respectively from losses arising from insect infestation during field storage in traditional crib for six months.

2. Materials and methods

2.1. Plant materials and test insects

Test plant materials were used as powder, water and ethanol extracts against test insect species. Each of these formulations were prepared following the procedure used by Denloye et al. (2007). *Callosobruchus maculatus, S. zeamais* and *T. castaneum,* were obtained from cultures maintained at Nigerian Stored Product Research Institute (NSPRI), Abule-Oja, Lagos, Nigeria. Fresh experimental cultures were prepared from the original stocks as described by Denloye et al. (2007).

2.2. Bioassays

Powder, aqueous and ethanolic extracts of *Z. zanthoxyloides* were respectively screened using the method of Denloye et al. (2007) to detect bioactivity of the test materials against each of the insect species cultured for the present study. Twenty active 1 to 3-d-old *C. maculatus* adults (mixed sexes), 1 to 7-d-old *S. zeamais* (mixed sexes) or 1 to 7-d-old adult *T. castaneum* (mixed sexes) were separately exposed to grains treated with each formulation and mortality assessments made every 24 h after treatment for 2 d.

Test plant materials were retested against the test insect species in more elaborate bioassays to measure acute toxicity levels dependent on 48 h LC_{50} and LC_{95} values as described by Denloye et al. (2007). For these series of experiments, 20 unsexed adult insects, and same age ranges given earlier were exposed per replicate of each treatment and the controls. For the test powder against each insect species the admixture concentrations used were 0.125 to 8.00 g kg^{-1} grain. For aqueous extracts the grains were dipped in extracts of 0.10 to 1.60 g kg^{-1} concentrations and for ethanolic extract 0.01 to 0.032 g L^{-1} concentrations were used.

Forty undamaged cowpea grains were treated by dipping in concentrations of 0.01, 0.02, 0.04, 0.08 and 0.16 g L^{-1} ethanol extract in four replicates. Treated grains were allowed to drain on filter paper for 5 min before transferring into bioassay containers. Mortality of exposed *C. maculatus* was assessed every 24 h. Several sets of 40 cowpea seeds treated at these concentrations with untreated seeds as controls were prepared at the same time. For each set of treated seeds and controls, bioassays were started off by introducing 10 unsexed 1 to 3-d-old adult *C. maculatus* to 1-, 12, 24, 96, 168 and 136 h predetermined post-treatment time intervals after treatment. Each treatment and controls were replicated four times. Insect mortality was assessed every 24 h for 2 d.

Cowpea seeds were treated at two concentrations (0.025 g L^{-1} and 0.10 g L^{-1}) of *Z. zanthoxyloides* ethanolic extract by dipping. Four 0 to 3-d-old adult *C. maculatus* (2 ♂, 2 ♀) were then confined for seven days with 20 treated or untreated cowpea seeds in clean glass Petri dishes securely covered. All treatments including control seeds that were dipped in ethanol only were replicated five times. Adults that died within the 7-d exposure periods were removed and replaced with other insects of the same age and sex. At the end of the 7-d oviposition period, all adults were removed. The seeds were inspected for eggs and were counted under binocular microscope (x8 objective). The seeds bearing eggs were then kept in covered vials and monitored daily for adult emergence. Emerging adults were counted and

removed from each treatment daily for 14 d after the first emergence was observed to prevent overlap of generations.

Similar experiments as described above were carried out, however, in this case a series of grains were treated by dipping in 0.025 g L^{-1} and 0.20 g L^{-1} of ethanolic extracts of *Z. zanthoxyloides* and ethanol for control. Two 0 to 3-d-old adult *C. maculatus* (1 ♂ and 1 ♀) were then confined with each set of extract-treated or ethanol-treated cowpea grains for 24 h, after which the pair of *C. maculatus* was transferred onto another batch of treated cowpea using the same extract concentration or control. The process was repeated every 24 h for 7 d. Each treatment batch was replicated four times. The number of eggs laid per day in each replicate of the treatments and control grains were counted daily under a binocular microscope (x8 objective).

2.3. Protectant evaluation

Disinfested cowpea or maize grains (5.0 kg) were measured into jute bags and manually admixed with powdered *Z. zanthoxyloides* at 2.0 g kg^{-1} and untreated controls. Each jute bag with the treated or untreated grain was securely tied and stored in traditional crib with thatched roof in an open field for 180 d. There were four replicate bags of treated or untreated grain arranged randomly with one replicate of each treatment on each of the four layers per crib. The assumption was that bags of grain left in cribs in the field are liable to infestation by the appropriate storage insect pest over time. To evaluate the results of the series of experiment, 100-g samples of cowpea or maize were taken from each bag, once every 30 d and assessed for insect damage according to Odeyemi and Daramola (2000).

2.5. Data analyses

Toxicological dose-response data involving mortality of test insect were analyzed by probit analysis (Finney, 1971) after correcting for mortality in control based on a computer program to obtain the median lethal concentration (LC_{50}) and the corresponding LC_{95}. Analysis of variance (ANOVA) was used to compare treatment means where the design fitted the requirements dependent on Statistical Package for Social Sciences (SPSS) version 11.0 (SPSS, 2001). Post-hoc analysis was carried out only where there was a significant difference at the 5% ($P < 0.05$) level of significance by comparing pairs of means based on Least Significant Differences (LSD). Monthly weight loss in each treatment and control was determined from 100-g batches of grains in each jute bag after Odeyemi and Daramola (2000) as follows:

$$\text{Percent weight loss} = \frac{(W_u \times N_d) - (W_d \times N_u) \times 100}{W_u (N_d + N_u)}$$

Where W_u = Weight of undamaged grains

N_u = Number of undamaged grains

W_d = Weight of damaged grains

N_d = Number of damaged grains

3. Results

The results show all test formulations were toxic to each of the three insect species exposed on treated grains (Table 1). Based on 48 h LC_{50} values, more detailed bioassays showed that the powder was significantly more toxic to *S. zeamais* (0.012 g kg^{-1}) than to either *T. castaneum* (0.41 g kg^{-1}) or *C. maculatus* (0.50 g kg^{-1}) (Table 2). The LC_{50} values also shows that the ethanolic extract was significantly more toxic to each of the test insect species than the aqueous extract. The ethanolic extract was however significantly less toxic to *T. castaneum* than to either *C. maculatus* or *S. zeamais* respectively (Table 2). Tests also showed further that the toxicity of the ethanolic extract of *Z. zanthoxyloides* remained constant for 336 h, the LC_{50} values remaining fairly constant at about 0.010 g/kg^{1} (Figure 1).

Table 1 Mortality of test insects on grains treated with *Z. zanthoxyloides*

Formulation	Test Insect	Mortality		
Powder	**Species**	**0.00 g/kg**	**1.00 g/ kg**	**20.00 g/kg**
	C. maculatus	0.00 (0.71) a	83.35 (9.16) a	100.00 (10.02) b
	S. zeamais	0.00 (0.71) a	83.35 (9.16) a	93.30 (9.69) b
	T. casteneum	0.00 (0.71) a	71.65 (8.49) b	100.00 (10.02) b
Aqueous Extract		**0.00 g/L**	**1.00 g/L**	**10.00 g/L**
	C. maculatus	0.00 (0.71) a	6.65 (2.67) b	25.00 (5.05) b
	S. zeamais	0.00 (0.71) a	5.00 (2.35) b	20.00 (4.53) c
	T. casteneum	0.00 (0.71) a	0.00 (0.71) a	25.00 (5.05) b
Ethanolic Extract		**0.00 g/L**	**1.00 g/L**	**10.00 g/L**
	C. maculatus	0.00 (0.71) a	60.00 (7.78) b	90.00 (9.51) c
	S. zeamais	0.00 (0.71) a	63.35 (7.99) b	95.00 (9.77) c
	T. casteneum	0.00 (0.71) a	90.00 (9.51) b	100.00 (10.02) c

Each datum is a mean of three replicates. Values in parentheses are square root ($\sqrt{x} + 0.5$) transformed. Column transformed means for each test plant extract bearing the same superscripts are not significantly different by Least Significant Difference (LSD) following Analysis of Variance (ANOVA); $P < 0.05$.

Table 2 Acute toxicity of *Zanthoxyllum zanthoxyloides* to test insects

Formulation	Test Insect		95 % Confidence limits	Regression Equation	Degree of Freedom	Slope ± Standard Error
Powder	**Species**	**48 hr LC_{50} (g/kg)**				
	C. maculatus	0.050	0.007 – 0.228	Y = 2.77 + 2.124x	4	2.12 ± 0.75
	S. zeamais	0.012	0.0 – 0.055	Y = 1.54 + 0.803x	4	0.803 ± 0.042
	T. castaneum	0.041	0.007 – 0.111	Y = 1.806 + 1.303x	4	1.303 ± 0.088
Aqueous Extract		**48 hr LC_{50} (g/L)**				
	C. maculatus	0.834	0.633 – 1.042	Y = 0.127 + 1.605x	3	1.605± 0.034
	S. zeamais	0.334	0.26 – 0.427	Y = 0.586 + 1.232 x	3	1.232 ± 0.026
	T. castaneum	0.383	0.296 – 0.496	Y = 0.486 +1.168x	3	1.168 ± 0.025
Ethanolic Extract		**48 hr LC_{50} (g/L)**				
	C. maculatus	0.021	0.012 – 0.022	Y = 2.263 + 1.476x	3	1.476 ± 0.024
	S. zeamais	0.035	0.020 – 0.041	Y = 1.567 + 1.021 x	3	1.021 ± 0.021
	T. castaneum	0.085	0.029 – 0.096	Y = 0.486 +1.168x	3	1.021 ± 0.021

LC_{50} values with no overlap in their 95 % confidence limits are significantly different ($p < 0.05$).

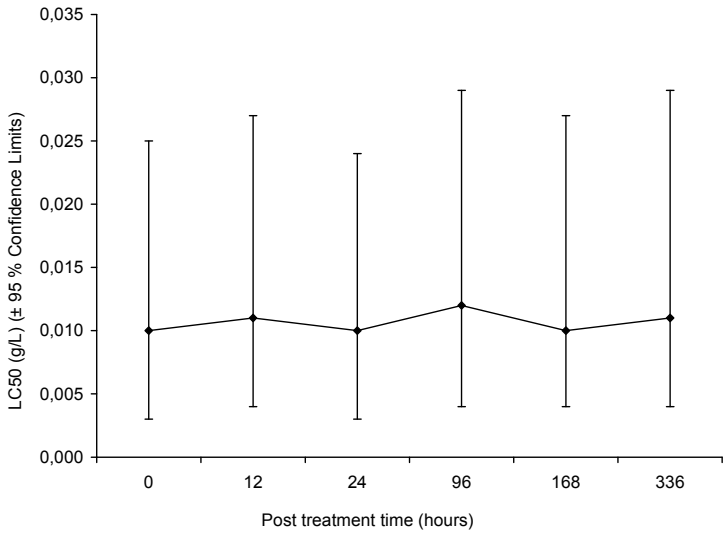

Figure 1 Persistence of *Z. zanthoxyloides* ethanol extract toxicity in treated cowpea grains

Treatment of cowpea grains with *Z. zanthoxyloides* ethanol extract caused significant reduction in the number of eggs laid by *C. maculatus,* but not a corresponding increase in adult emergence (Table 3a). Tests also showed that the test ethanolic extract treatment had no effect on the daily oviposition rate of *C. maculatus* at the low treatment (0.025 g kg^{1}) but it delayed the commencement of egg laying for three days at the high concentration with a significantly reduced number of eggs laid (Table 3b).The experiments on weight loss of treated grains showed that there was no weight loss for 180 d in treated cowpea and 150 d in treated maize, whereas the untreated grains had 3.12 g and 5.04 g for weight losses for maize and cowpea, respectively, after 180 d of storage (Table 4).

Table 3a Effect of *Zanthoxyllum zanthoxyloides* on oviposition and progeny production of *C. maculatus*

Treatment	(g/L)	Mean number of Eggs laid (± SE)	Mean adult emergence (± SE)	Mean percent adult emergence (± SE)
Z. zanthoxyloides	(0.00)	93.30 ± 3.46 a	41.00 ± 2.58	43.95 ± 4.76
	(0.025)	53.00 ± 1.63 b	18.00 ± 3.16	33.88 ± 5.28
	(0.10)	21.00 ± 4.57 c	7.00 ± 2.45	32.86 ± 4.99

Column means bearing same superscripts are not significantly different (P > 0.05) by Least Significant Difference (LSD) Test. SE = Standard Error

Table 3b Effect of *Z. zanthoxyloides* on daily oviposition by *C. maculatus* in treated cowpea seeds

Extracts	Concentration (g/L)	Oviposition days							
		1	2	3	4	5	6	7	Total
Z. zanthoxyloides	0.0	2	4	10	10	9	5	3	43 a
	0.025	1	5	7	10	10	2	2	37 a
	0.20	0	0	0	3	2	1	1	7 b

Each datum is a mean of four replicates. Means for total number of eggs laid on seeds treated with each test plant extract bearing the same superscripts are not significantly different by Least Significant Difference (LSD) following Analysis of Variance (ANOVA); P < 0.05.

Table 4 Weight loss of grains protected by treatment with *Z. zanthoxyloides*

	Weight loss (g) in treated grains			
Post-treatment	Maize		Cowpea	
Time (Days)	Control	Treated grains	Control	Treated grains
0	0.00 ± 0.00	0.0 ± 0.00	0.00 ± 0.00	0.0 ± 0.00
30	0.00 ± 0.00	0.0 ± 0.00	0.00 ± 0.00	0.0 ± 0.00
60	0.02 ± 0.00	0.0 ± 0.00	0.03 ± 0.001	0.0 ± 0.00
90	1.44 ± 0.35	0.0 ± 0.00	1.79 ± 0.23	0.0 ± 0.00
120	1.80 ± 0.35	0.0 ± 0.00	2.21 ± 0.42	0.0 ± 0.00
150	2.47 ± 0.29	0.0 ± 0.00	2.83 ± 0.19	0.0 ± 0.00
180	3.12 ± 0.44	0.05 ± 0.01	5.04 ± 1.15	0.0 ± 0.00

Each datum is a mean of four replications.

4. Discussion

The biological activity of *Z. zanthoxyloides* was investigated in laboratory bioassays and semi-field trials to evaluate the potentials of the plant as a source of insecticide for the protection of stored grains from attack by *C. maculatus, S. zeamais* and *T. castaneum*. On the basis of properties required in chemicals for controlling insects feeding on grains such as toxicity to adults and oviposition suppression the test plant materials have shown some appreciable potential under these parameters. In this study *Z. zanthoxyloides* powder and ethanolic extract demonstrated toxicity against the adults and the extract reduced oviposition by *C. maculatus*. These findings agree with a similar study by Ogunwolu and Odunlami (1996), where root bark powder of *Z. zanthoxyloides* was toxic to the adults and caused fewer eggs to be laid with a corresponding smaller number of emerged adults in cowpea seeds treated with the test powder compared with the control. In the present study, the ethanolic extract remained toxic in laboratory tests against *C. maculatus* for 336 h without losing its potency against the insects. The practicality of using the test plant

as grain protectant was demonstrated in this study when no weight loss of treated grains was recorded for at least 150 d (5 months).

Based on these results, the plant materials can similarly be used by subsistence farmers to protect stored cowpea and maize grains against *C. maculatus* and *S. zeamais* in small storage systems in Nigeria and other African countries such as Malawi and Benin Republic (Delobel and Malonga, 1987; Terpondju et al., 2002). The toxicity and oviposition suppression activity of the test plant products in this study were caused by the bioactivity of their chemical constituents and physical action of the formulations. While there is need to isolate and identify the chemical constituents of the test plant materials the physical actions may be explained. For instance the bioactive constituents of the plant materials may be more available in the ethanolic extract since and be responsible for the extract's higher toxicity. The powder formulations used in this study could have caused insect mortality due to their physical action on respiration through blockage of the spiracles of the test insects. Although there is no direct evidence of this in our test earlier studies have shown that there is a direct relationship between particle size of plant powders and insect mortality in treated grains (Ogunwolu and Odunlami, 1996; Ofuya and Dawodu, 2004). In addition, fine particle size such as the one used in this study helps to even the distribution of powders on the surface of seeds and the walls of the storage container, thus increasing their possibility of getting in contact with the insects and killing them. In addition, plant powders cause abrasion of insect cuticle and lead to water loss (Sousa et al., 2005), thus causing stress and eventual death. Our study shows *Z. zanthoxyloides* has high potential for use as protectant of maize and cowpea grains in small scale storage systems in Nigeria.

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