

Full Length Research Paper

Biological effect of ethanolic extract fractions of *Ricinodendron heudelotii* (Baill) Pierre ex Pax against *Sitophilus zeamais* Motschulsky and *Callosobruchus maculatus* Fabricius on stored grains

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Ethanolic extract of dried leaves of *Ricinodendron heudelotii* was partitioned between equal volumes of hexane, ethylacetate, chloroform, butanol and water to obtain the various fractions. These fractions were assessed in the laboratory against *Sitophilus zeamais* and *Callosobruchus maculatus* on stored maize and cowpea, respectively. 100 g each of maize and cowpea grains were treated with the extract fractions to test for contact toxicity, damage assessment and progeny production. Furthermore, contact toxicity on filter paper and toxicity by topical application as well as repellency test were also carried out. Results obtained showed a significant ($P < 0.05$) insect mortality when the fractions were applied topically and by grain treatment. There was also a significant reduction in damage caused while progeny development was very low. The extract fractions significantly repelled the 2 insect species with an overall repellency of 48%. This work recommends the incorporation of *R. heudeotii* into storage pest management systems.

Key words: *Ricinodendron heudelotii*, toxicity, repellency, stored grains, *Callosobruchus maculatus*, *Sitophilus zeamais*.

INTRODUCTION

Grains constitute the most important staple foodstuff for the evergrowing population in the tropics. As in field crops, a wide range of insect pests attack stored products with the commonest among them being beetles and moths (Obeng - Ofori et al., 1997).

Insect pest damage to stored grains results to major economic losses to farmers throughout the world. In most tropical countries, post harvest losses of cereals and pulses due to attack by insect pests have been estimated at 20 - 30% (Dick, 1988). The extent of stored grain losses vary according to insect species and come with serious economic consequences, thus threatening food security. For instance, *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae) is known to cause up

to 100% loss of stored cowpea and estimates have shown that over 30 million US dollars (about 5 billion naira) is lost as a result of cowpea damage in Nigeria (Jackai and Daoust, 1986). In Ghana, over 20% of about 300,000 tonnes of maize stored is lost to *Sitophilus zeamais* Motschulsky (Coleoptera:Curculionidae)(Tindall, 1983) while 20% of maize cobs were found already infested with weevils at the time of harvest in Nyanza District of Kenya (Nyambo, 1993).

As a measure to curtail the infestation of stored products by insect pests, farmers have largely depended on the use of synthetic insecticides. This has led to the development of insect strains resistant to insecticides while toxic residues on stored grains constitute health hazards to grain handlers and consumers aside from the problem of persistence in the environment. Apart from the above, synthetic chemicals are expensive, erratic in supply due to foreign exchange constraints and cost – benefit wise often not economical to use by resource

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poor farmers (Niber, 1994; Udo, 2005). These problems therefore call for new alternative control measures and presently, attention has been turned to botanicals. Most botanicals are broad spectrum in action, safe to the environment and cause few hazards to man and other animals. This gave impetus to the screening of *Ricinodendron heudelotii* (Baill) Pierre ex Pax for insecticidal properties against *S. zeamais* and *C. maculatus* on stored maize and cowpea, respectively.

MATERIALS AND METHODS

Insects

S. zeamais and *C. maculatus* were collected from infested stock of grains at the Uyo (South-Eastern Nigeria) main market and reared on whole maize and cowpea grains in the Crop Protection Laboratory, University of Uyo, Nigeria. After 2 weeks of oviposition, the parent adults were removed by sieving and progeny emerging were re-cultured. Culture conditions were $28 \pm 2^\circ\text{C}$, 65% relative humidity and 12 hL: 12 hD photoregime and all experiments were carried out under same conditions.

Collection of plant materials and preparation of extract fractions

2 kg of the leaves of *R. heudelotii* were collected from Uyo metropolis, Nigeria and air-dried in the laboratory for about 2 weeks. The dried leaves were ground and soaked in 95% Ethanol in glass jars and left for 72 h. The mixture was filtered and the filtrate was evaporated to dryness in a vacuo using rotary evaporator (Udo et al., 2004). The crude extract was then dissolved in 1 L of distilled water and subjected to partitioning using hexane, chloroform, ethyl acetate and butanol to obtain the extract fractions. The partitioned fractions were then concentrated to dryness in vacuo using rotary evaporator and later re-dissolved in distilled water and used for the various bioassays.

Contact toxicity by topical application

40 adult unsexed insects in batches of 10 each of *S. zeamais* and *C. maculatus* were chilled in the refrigerator for 3 min to reduce their activity (mobility). Thereafter, they were transferred into petri dishes (11.0 cm diameter) lined with moist filter paper (Obeng-Ofori et al., 1998). Insects were picked individually and with the aid of a micropipette, 20 $\mu\text{l/ml}$ of various extract fractions were applied to the dorsal surface of the thorax of each insect. Distilled water was applied to the control insects and each treatment was replicated four times. Mortality was recorded after 1 h and up to 48 h.

Toxicity of extract fractions in grains

Toxicity of the different extract fractions on maize and cowpea grains was tested in the laboratory by applying 400 mg/kg to 50 g of grains in a 200 ml plastic cup. The extract fractions were allowed to dry for 30 min. 10 pairs each of *S. zeamais* and *C. maculatus* were introduced into the plastic cups and covered with white muslin cloth held in place with rubber bands. The control was treated with distilled water only. Mortality was recorded after 24 h and up to 96 h. Insects were presumed dead on failure to respond to three probings with a blunt probe after a 5 min recovery time.

Contact toxicity on filter paper

A Whatman No.1 filter paper (10.9 cm diameter) was placed in a glass petri dish (11.0 cm diameter) and 200 $\mu\text{l/ml}$ of each extract fraction was applied separately to the filter paper and left for about 30 min to dry off (Obeng-Ofori et al., 1998; Udo et al., 2004). 10 adults each of *S. zeamais* and *C. maculatus* were introduced into each dish, respectively. Controlled dishes were treated with distilled water only and each treatment was replicated four times. Insect mortality was recorded after 24 h and up to 96 h.

Damage assessment

100 g of maize and cowpea grains were treated with 400 mg/kg of each extract fraction and 20 adults each of *S. zeamais* and *C. maculatus* were introduced into treated and control grains. Control grains were treated with distilled water and each treatment was replicated four times and left undisturbed for 4 weeks. Samples of 100 grains were taken from each cup and the number of damaged grains (grains with characteristic holes) and undamaged grains were counted and weighed. The percent weight loss was computed using the method of FAO (1985).

Progeny production

10 g of pre-equilibrated maize and cowpea grains were treated with 400 mg/kg of each extract fraction and allowed to dry for 3 h after which 20 adults each of *S. zeamais* and *C. maculatus* were introduced into the grains while the control was treated with distilled water. The containers were covered with white muslin cloth and held in place with rubber bands. The experiment was replicated four times and left undisturbed for 5 weeks and number of insects emerging was counted.

Repellency test

Repellency of the extract fractions was assessed in a choice bioassay method using baked wheat cakes (Udo et al., 2004). 100 g of wheat flour was mixed with one litre of water and the resultant dough made into small round balls of about 10 g each. The cakes were baked in the oven at 40°C for 6 h. 2 baked cakes were treated with each extract fraction at the rate of 100 mg/kg. 2 control cakes were treated with distilled water only. Treated and control cakes were air-dried for 1 h before introducing 10 adults of each insect species into the center of petri dishes containing the cakes. The treated and control cakes were separated by a space in the center of the petri dish and each treatment was replicated four times. Number of insects present on the control (N_c) and treated (N_t) cakes was recorded after 1 h and up to 6 h. Percent repellency was computed as:

$$PR = \frac{N_c - N_t}{N_c + N_t} \times 100\%$$

Where: PR = percent repellency

N_c = insect number present on control strip

N_t = insect number present on treated strip

Negative PR values were treated as zero

Data analyses

The data obtained were subjected to analysis of variance (ANOVA)

Table 1. Toxicity of extract fractions of *R. heudelotii* applied topically against *S. zeamais* and *C. maculatus*.

Extract fractions 20 µl/ml	Mean percent mortality hours after treatment				
	24	48	72	96	control
<i>S. zeamais</i>					
Ethyl acetate	65 ^a ± 0.50	80 ^a ± 1.15	80 ^a ± 1.15	85 ^a ± 0.96	0 ^b ± 0.00
Chloroform	75 ^b ± 0.50	85 ^{ab} ± 0.50	90 ^a ± 0.50	95 ^a ± 0.50	0 ^b ± 0.00
Hexane	95 ^a ± 0.50	95 ^a ± 0.50	100 ^a ± 0.00	100 ^a ± 0.00	0 ^b ± 0.00
Butanol	95 ^a ± 0.00	100 ^a ± 0.00	100 ^a ± 0.00	100 ^a ± 0.00	0 ^b ± 0.00
Aqueous	0 ^{NS} ± 0.00	0 ^{NS} ± 1.00	10 ^{NS} ± 1.00	15 ^{NS} ± 0.96	0 ^{NS} ± 0.00
<i>C. maculatus</i>					
Ethyl acetate	90 ^a ± 0.00	100 ^a ± 0.00	100 ^a ± 0.00	100 ^a ± 0.00	0 ^b ± 0.00
Chloroform	85 ^{ab} ± 0.96	100 ^a ± 0.00	100 ^a ± 0.00	100 ^a ± 0.00	0 ^b ± 0.00
Hexane	95 ^a ± 0.50	100 ^a ± 0.00	100 ^a ± 0.00	100 ^a ± 0.00	0 ^b ± 0.00
Butanol	95 ^a ± 0.50	100 ^a ± 0.00	100 ^a ± 0.00	100 ^a ± 0.00	0 ^b ± 0.00
Aqueous	15 ^b ± 0.50	25 ^{ab} ± 0.50	30 ^a ± 0.58	30 ^a ± 0.58	0 ^c ± 0.00

Means of 4 replicates of 10 insects each. Means for each fraction and in the same row followed by different letter(s) are significantly different ($p < 0.01$) NS = Not significant.

Table 2. Contact toxicity of extract fractions of *R. heudelotii* applied on grains against *S. zeamais* and *C. maculatus*.

Extract fractions 400 mg/kg	Mean percent mortality at different times (hour) after treatment					
	24	48	72	96	Control	LSD
<i>S. zeamais</i>						
Ethyl acetate	3 ± 0.50	5 ± 1.10	8 ± 0.96	10 ± 0.82	0 ± 0.00	NS
Chloroform	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	NS
Hexane	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	NS
Butanol	0 ± 0.00	1 ± 0.25	4 ± 1.50	5 ± 1.83	0 ± 0.00	NS
Aqueous	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	NS
<i>C. maculatus</i>						
Ethyl acetate	20 ± 0.82	60 ± 0.82	73 ± 0.96	78 ± 1.50	0 ± 0.00	14.30
Chloroform	23 ± 0.96	43 ± 1.26	50 ± 1.15	58 ± 1.50	0 ± 0.00	17.20
Hexane	23 ± 1.26	33 ± 1.71	35 ± 0.58	43 ± 0.58	0 ± 0.00	23.80
Butanol	10 ± 2.06	10 ± 2.16	10 ± 2.16	30 ± 0.57	0 ± 0.00	NS
Aqueous	6 ± 0.68	35 ± 1.32	61 ± 1.50	78 ± 0.86	0 ± 0.00	19.20

Means of 4 replicates of 10 insects each. LSD test ($P < 0.05$) NS = Not significant.

according to procedures of Statistical Analysis System (SAS, 1999).

RESULTS

Contact toxicity by topical application

Toxicity of the various extract fractions applied topically to *S. zeamais* and *C. maculatus* is summarized in Table 1. There was a significant ($P < 0.05$) mortality of 100% observed in all the extract fractions against *C. maculatus* 96 h after treatment except for the aqueous fraction which recorded a mortality of 30%. In the case of *S. zeamais*, hexane and butanol fractions produced 100%

insect mortality after 96 h of treatment. Ethyl acetate and chloroform fractions recorded 85 and 50% mortality of *S. zeamais*, respectively after 96 h of treatment. However the aqueous fraction failed to produce any significant effect on *S. zeamais*.

Toxicity of the extract fractions in grains

The toxicity of extract fractions of *P. heudelotii* applied on grains against *C. maculatus* showed a significant difference ($P < 0.05$) in mortality over the control (Table 2). The ethyl acetate and aqueous fractions gave the highest mortality of 78% after 96 h of treatment.

Table 3. Contact toxicity of extract fractions of *R. heudelotii* applied on filter paper against *S. zeamais* and *C. maculatus*.

Extract fractions 200 µl/ml	Mean percent mortality at different times (hour) after treatment					
	24	48	72	96	control	LSD
<i>S. zeamais</i>						
Ethyl acetate	0 ± 0.00	5 ± 0.00	5 ± 0.00	10 ± 0.00	0 ± 0.00	NS
Chloroform	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	NS
Hexane	5 ± 0.00	5 ± 0.00	5 ± 0.00	5 ± 0.00	0 ± 0.00	NS
Butanol	0 ± 0.00	5 ± 0.00	5 ± 0.00	5 ± 0.00	0 ± 0.00	NS
Aqueous	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	NS
<i>C. maculatus</i>						
Ethyl acetate	0 ± 0.00	0 ± 0.00	20 ± 0.82	35 ± 1.50	0 ± 0.00	23.60
Chloroform	0 ± 0.00	20 ± 0.82	25 ± 0.50	50 ± 0.58	0 ± 0.00	15.60
Hexane	0 ± 0.00	15 ± 0.96	20 ± 1.41	25 ± 1.50	0 ± 0.00	NS
Butanol	5 ± 0.50	15 ± 0.96	20 ± 0.82	30 ± 1.29	0 ± 0.00	NS
Aqueous	20 ± 2.16	50 ± 5.46	68 ± 4.57	90 ± 0.62	0 ± 0.00	6.07

Means of 4 replicates of 20 insects each. LSD test ($P < 0.05$) NS = Not significant.

Table 4. Effect of extract fractions of *R. heudelotii* on damaged caused by *S. zeamais* and *C. maculatus*.

Extraction fractions	Mean percent weight loss	
	<i>S. zeamais</i>	<i>C. maculatus</i>
200 mg/kg		
Ethyl acetate	0.29 ± 0.19	0.68 ± 0.46
Chloroform	0.23 ± 0.10	0.60 ± 0.23
Hexane	0.18 ± 0.13	0.62 ± 0.49
Butanol	0.44 ± 0.27	0.85 ± 0.52
Aqueous	0.79 ± 0.61	0.50 ± 0.21
Control	7.35 ± 1.78	6.44 ± 2.94
LSD	1.16	1.87
400 mg/kg		
Ethyl acetate	0.27 ± 0.28	0.15 ± 0.18
Chloroform	0.14 ± 0.17	0.39 ± 0.41
Hexane	0.33 ± 0.46	0.49 ± 0.33
Butanol	0.73 ± 0.39	0.79 ± 0.61
Aqueous	0.49 ± 0.20	0.99 ± 0.51
Control	4.79 ± 1.38	9.46 ± 4.08
LSD	0.95	2.54

LSD test ($P < 0.01$).

However, the same extract fractions applied on grains against *S. zeamais* produced no significant mortality even though ethyl acetate and butanol fractions showed some

level of activity.

Contact toxicity on filter paper

Results of the effect of the various extract fractions of *P. heudelotii* applied on filter paper is summarized in Table 3. No significant effect was observed against *S. zeamais* but ethyl acetate, chloroform and aqueous fractions significantly ($P < 0.05$) affected *C. maculatus*. However, *S. zeamais* were observed to be hanging on the underside of lids thus avoiding treated surfaces.

Damage assessment

There were significant differences ($P < 0.01$) amongst the extract fractions of *R. heudelotii* in reducing damage caused by the beetles (Table 4). Hexane fraction applied at 200 g/kg recorded a minimal weight loss of 0.18% in stored maize.

However, when the dosage was raised to 400 mg/kg ethyl acetate fraction protected cowpea grains stored with an observed weight loss of 0.15%. Better protection of both maize and cowpea grains were achieved from the different extract fractions compared with the untreated control (Table 4).

Progeny production

The extract fractions of *P. heudelotii* significantly ($P 0.01$) affected the F₁ generation produced by *S. zeamais* and *C. maculatus*, respectively (Table 5). The butanol

Table 5. Effect of extract fractions of *R. heudelotii* on F₁ Progeny produced by *S. zeamais* and *C. maculatus*.

Extraction fractions	Mean percent weight loss	
	<i>S. zeamais</i>	<i>C. maculatus</i>
200 mg/kg		
Ethyl acetate	33.00 ± 2.16	37.75 ± 26.39
Chloroform	29.25 ± 8.96	23.50 ± 13.99
Hexane	34.50 ± 3.11	21.25 ± 5.62
Butanol	0.44 ± 0.27	0.85 ± 0.52
Aqueous	19.50 ± 9.331	25.00 ± 5.48
Control	46.00 ± 10.80	62.50 ± 12.39
LSD	11.29	20.88

Means (± SEM) of four replicates of 20 insects each LSD test (P < 0.01)

Table 6. Mean percent repellency (PR) values for extract fractions of *R. heudelotii* against the two insect species in a choice test.

Extraction fractions	Mean % Repellency Values	
	<i>S. zeamais</i>	<i>C. maculatus</i>
Ethyl acetate	55	35
Chloroform	62	58
Hexane	44	61
Butanol	24	52
Aqueous	53	34
Overall PR	48	48
LSD	26.42	NS

Means of 4Replicates of 10 Insects each. LSD test (P < 0.01). NS = Not significant.

fraction inhibited the number of F₁ progeny produced by *S. zeamais* more than the other fractions while the hexane fraction gave the lowest number of F₁ progeny recorded by *C. maculatus*.

Repellency bioassay

The different extract fractions of *P. heudelotii* showed varying levels of repellency to the two insect species (Table 6). *S. zeamais* was significantly repelled with an overall repellency of 48% with chloroform fraction recording the highest repellent effect of 62%. However, no significant repellent effect was observed against *C. maculatus*.

DISCUSSION

Toxicity of the different extract fractions evaluated against the two insect species showed some level of activity against them. A very high potency was observed when

the extract fractions were applied topically to both insect species. Topical application facilitated direct contact of toxicants or active ingredients in *P. heudelotii* with the insects' bodies. Similar results have been obtained by earlier workers who screened natural biopesticide against various arthropod pests (Okonkwo and Okoye, 1996; Adedire and Ajayi, 1996). Furthermore, a significant contact action on filter paper treated with *R. heudelotii* fractions against *C. maculatus* is noteworthy and strongly indicates the presence of insecticidal properties in the plant. *C. maculatus* adults are good fliers and the little contact on treated surface produced significant mortality as observed in the ethyl acetate, chloroform and aqueous fractions. Contact toxicity on treated grains was more pronounced in *C. maculatus* probably because of their soft bodies making it possible for easy penetration of toxicants, compared with their *Sitophilus* counterparts. It could also be argued that *S. seamais* avoided treated grains thus reducing contact and possible assimilation of toxicants as the insects were observed hanging on the underside of muslin cloths. This again might confirm repellent properties in *R. heudelotii* as secondary plant chemicals are known to attract or repel insects and influence their locomotion, oviposition and feeding behaviour (Obeng-Ofori et al., 1998).

The significant reduction in damage caused by the 2 insect species to stored grains coupled with high insect mortality could be attributed to the presence of toxic secondary metabolites. It has been reported that some secondary metabolites may act both as insecticides and antifeedants as observed for rotenone against *T. castaneum* (Nawrot et al., 1989).

The extract fractions of *R. heudelotii* were effective in reducing the population of F₁ generation of both *S. zeamais* and *C. maculatus* in treated grains. *C. maculatus* was observed to produce lower number of F₁ progeny probably because eggs of *C. maculatus* are laid on the seed coat thus exposing them to direct contact with toxic secondary metabolites. The significant reduction in F₁ progeny confirms the possible presence of ovicidal and larvicidal constituents in the plant (Tanzubil, 1991). It would also appear that progeny inhibition of *S. zeamais* and *C. maculatus* in treated grains could arise from the inability of young adult insects to chew through the seed coats of treated grains due to the presence of phenolic compounds (Wongo, 1998).

The significant repellent action of the extract fractions against the 2 insect species showed they reacted to secondary metabolites. However, different species react differently to plant metabolites with some being attracted while others are repelled (Obeng-Ofori et al., 1998), thus the repellent action of the extract fractions strongly suggest the presence of antifeedant properties (Udo et al., 2004).

The results obtained in the study suggest good potential for the use of *R. heudelotii* in storage pest management systems, as botanicals are broad spectrum

in action and safe to the environment with fewer hazards to man and other mammals.

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