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Biological Activity of Ethanolic Extract Fractions of *Dracaena arborea* Against Infestation of Stored Grains by Two Storage Insect Pests

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Abstract: As part of on-going efforts to use eco-friendly alternatives to chemical pesticides, ethanolic extract of dried leaves of *Dracaena arborea* (Willd.) Link (Dragon tree; Dracaenaceae) dissolved in distilled water and partitioned between equal volumes of n-hexane, chloroform, ethyl acetate and butanol was assessed in the laboratory against infestation by *Sitophillus zeamais* Motsch. and *Callosobruchus maculatus* Walp. in stored maize and cowpea, respectively. One hundred grams each of maize grains and cowpea seeds were treated with 400 mg kg^{-1} of each extract fraction to evaluate contact toxicity, damage assessment, effect on eggs and immature stages and progeny production in both insect species. Contact toxicity by topical application, toxicity upon filter paper application and repellency using area preference method were carried out on the two insect species. Results showed that the extract fraction caused significant ($p \leq 0.05$) mortality of both insect pests with a high residual contact activity against *S. zeamais*. Grain damage was significantly ($p \leq 0.01$) reduced, while progeny production and development of eggs within grains were inhibited. The extract fractions evoked a strong repellent action against *S. zeamais* but moderate action against *C. maculatus*. The full potentials of using extract fractions of *D. arborea* as grain protectant against infestation by insect pests is discussed.

Key words: *Dracaena arborea*, extract, *Callosobruchus maculatus*, *Sitophilus zeamais*, toxicity, repellency

INTRODUCTION

Insect pests, particularly storage pests continue to pose serious threat to food security if left unchecked. Enormous losses of up to 20-30% on stored products have been reported from storage insect pests like *Sitophilus zeamais* and *Callosobruchus maculatus* (Osuji, 1985). In a survey conducted at Nyanza District of Kenya, it was found out that approximately 20% of maize cobs were already infested with weevils at the time of harvest (Nyambo, 1993). Apart from direct damage to stored grains by insects, losses also occur as a result of contamination with insects faecal material and exuviae.

As a measure to contain the infestation of storage products by insect pests, farmers largely depend on the use of gaseous fumigants and residual chemical insecticides which are toxic to the consumer, causing health hazards to grain handlers and inducing widespread development of resistance in insect pests (Zettler and Cuperus, 1990). These problems, therefore, call for new alternative control measures and presently attention has been turned to botanicals because most of them are broad spectrum, safe to the environment and cause little or no hazards to man and other animals. *Dracaena arborea* (Willd.) Link (Dracaenaceae) is a woody stemmed tropical plant that grows up to 15 m high with a girth of 2.5 m and long broad leaves. It is usually utilized as boundary plants for demarcation, while there are claims to the presence in it of anti-parasitic and anti-fungal compounds (Okunji *et al*., 1996). Epidi *et al*. (2008) reported on the effect of leaf powder of *D. arborea* on aspects of the biology of *C. maculatus* and *S. zeamais*. In this study, ethanolic extract fractions of *D. arborea* are screened for their insecticidal properties against *Sitophillus zeamais* and *Callosobruchus maculatus*.

MATERIALS AND METHODS

Study period: The study was conducted between June 2005 and July 2007 in the laboratory of the Department of Crop Science, University of Uyo, Uyo, Nigeria.

Insects: *Sitophilus zeamais* and *Callosobruchus maculatus* were collected from infested stock of grains at the Uyo main market, Nigeria and reared on sterilized maize and cowpea grains, respectively. After 2 weeks

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of oviposition, the parent adults were removed using an impact test sieve with mesh size of 2 mm. Progeny that subsequently emerged were re-cultured and used for the various bioassays. Culture conditions were 28±2°C, 65-70% relative humidity and 12 h L, l2 h D light regime, while all experiments were carried out under same conditions.

Collection of plant materials and preparation of extract fractions: Two kilograms of leaves were collected from Uyo metropolis and air dried in the laboratory for one week. The dried leaves were ground and soaked in 95% ethanol in glass jars and left to stand for 72 h. The filtrate obtained was evaporated to dryness in a vacuo using rotary evaporator (Ofuya and Okuku, 1994). The crude extract was then dissolved in one liter of distilled water and subjected to partitioning using equal volumes of n-Hexane, chloroform, ethyl acetate and butanol to obtain the extract fractions. The partitioned fractions were then concentrated to dryness in vacuo using rotary evaporator, while the various residues obtained were dissolved in distilled water or acetone and were then used for the various bioassays.

Contact toxicity by topical application: Ten adult insects each of *S. zeamais* and *C. maculatus*, respectively were placed in Petri dishes lined with moist filter paper (Obeng-Ofori *et al.*, 1997). Insects were picked individually and for each extract 20 μl mL^{-1} was applied to the dorsal surface of the thorax with the aid of a micropipette. Distilled water was used for control insects and each treatment was replicated four times. Insects were examined daily for mortality within 96 h. Any insect that did not move or respond to a blunt probe applied a maximum of three times was considered dead.

Contact toxicity on filter paper: The method described by Obeng-Ofori *et al.* (1998) was adopted. A Whatman No.1 filter paper (10.9 cm diameter) was placed in a glass Petri dish (11.0 cm diameter) and 200 μl mL^{-1} of each extract fraction was applied separately to the filter paper and left for about 30 min to dry off. Ten adults of each insect species were introduced into each dish, respectively. Controls were treated with distilled water and each treatment replicated four times. Insect mortality was recorded after 24 h and up to 96 h. Insects were assumed dead if they remain immobile and also fail to respond to three probes with a blunt dissecting probe after a 5 min recovery period.

Toxicity of extract fractions applied on grains: Toxicity of the different extract fractions applied on maize grains for *S. zeamais* and on cowpea grains for *C. maculatus* was tested by applying 200 μl mL^{-1} to 50 g of grains in a 200 mL plastic cup. The extract fractions were allowed to dry up for 30 min after which ten pairs of each insect species were introduced into the plastic cups which were thereafter covered with white muslin cloth held in place by rubber bands. The control was treated with distilled water only. Mortality was recorded after 24 h and up to 96 h while insects were presumed dead on failure to respond to three probes with a blunt probe.

Level of protection offered by the extract fractions: One hundred grams of maize and cowpea grains, respectively were treated with 200 μl mL^{-1} of the different extract fractions and allowed to dry for 30 min. Ten pairs each of *S. zeamais* and *C. maculatus* were introduced into the 200 mL plastic cups and covered with white muslin cloth held in place with rubber bands and left undisturbed for four weeks. Control treatments had distilled water added. 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. Percent weight loss was calculated following the method of FAO (1985) as:

$$\text{Weight loss}(\%) = \frac{[UaN-(U+D)]}{UaN}$$

Where:
U = Weight of undamaged fraction in the sample
N = Total number of grains in the sample
Ua = Average weight of one undamaged grain
D = Weight of damaged fraction in the sample

Effect of extract fractions on eggs and immature stages: Batches of 200 g of sterilized maize and cowpea grains respectively were placed in 500 mL glass jar and the grains were infested with 100 adults each of *S. zeamais* and *C. maculatus* to allow for egg laying. The parent adults were removed after seven days. One day after removal of adults, four batches of 25g each of maize and cowpea were treated with 200 μl mL^{-1} of each extract fraction to test their effect on the eggs and immature stages. This process was repeated 1 week and 2 weeks after adult removal. Control was treated with distilled water and adults emerging subsequently were counted weekly following the removal of the parent adults (Udo *et al.*, 2004).

Progeny production: One hundred grams of pre-equilibrated maize and cowpea grains were treated with 200 μl mL^{-1} of each extract fraction and allowed to stand

for 30 min 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 cups were covered with white muslin cloth and held in place with rubber bands. The experiment was replicated four times and left to stand undisturbed for five weeks and number of insects emerging was counted.

Repellency test: Repellency of the extract fractions was assessed using area preference method as described by Obeng-Ofori *et al.* (1998). Test areas consisted of 22 cm Whatman No.1 filter papers cut into halves. The different extract fractions were applied at 200 μl mL^{-1} to a half filter paper disc as uniformly as possible using a pipette. The other filter paper halves were treated with distilled water only to serve as the control. The treated and control half discs were air dried for one h and full discs re-made by attaching treated and untreated halves with paper tapes. Each paper was placed in a Petri dish and 10 weevils introduced at the center of the paper and covered with perforated lids with white muslin cloth. Each treatment was replicated four times and the number of weevils present on the control (N_c) and the treated (N_t) strips were recorded after 30 min and up to 48 h. Percent Repellency (PR) values were computed as:

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

Where:

PR = Percent repellency
N_c = Number of insects present on control strip
N_t = Insect number present on treated strip. Negative PR values were treated as zero

RESULTS

Contract toxicity by tropical application: Toxicity of the various extract fractions of *D. arborea* applied topically to *S. zeamais* and *C. maculatus* is summarized in Table 1. There was a significant ($p \leq 0.05$) insect mortality with the aqueous fraction inducing l00% mortality in both insect species. Ethyl acetate fraction also produced 100% mortality in *C. maculatus* after 96 h of treatment. However, chloroform and n-hexane fractions showed the least toxicity to the two insect species.

Contact toxicity on filter paper: Results of the toxic effect of the different extract fractions of *D. arborea* applied on filter paper against the two insect species (Table 2) revealed different levels of activity against *S. zeamais*. Contact mortality of 80% was recorded from the ethyl acetate fraction against *S. zeamais* 96 h after treatment.

Table 1: Mean mortality (%) of *S. zeamais* and *C. maculatus* after topical application of various extract fractions of *D. arborea*

Extract fractions	Mean percent mortality hours after treatment (h)				
(20 μl mL^{-1})	24	48	72	96	LSD
S. zeamais					
Ethyl acetate	15±0.95	35±0.95	40±0.82	65±0.95	24.80
Chloroform	0±0.00	0±0.00	10±0.57	15±0.50	10.40
Hexane	0±0.00	0±0.00	5±0.50	10±0.57	NS
Butanol	70±1.73	75±1.89	75±1.89	85±1.50	47.40
Aqueous	95±0.05	100±0.00	100±0.00	100±0.00	7.40
C. maculatus					
Ethyl acetate	95±0.50	100±0.00	100±0.00	100±0.00	6.80
Chloroform	0±0.00	0±0.00	10±0.57	15±0.50	NS
Hexane	0±0.00	10±1.00	15±1.38	25±1.89	NS
Butanol	95±0.50	95±1.89	95±0.50	95±0.50	13.40
Aqueous	95±0.05	100±0.00	100±0.00	100±0.00	7.40

Means of four replicates of 10 insects each; LSD test ($p \leq 0.05$); NS = Non-significant

Table 2: Mean mortality (%) of *S. zeamais* and *C. maculatus* caused by contact toxicity of the various extract fractions of *D. arborea* (filter paper method)

Extract fractions	Mean percent mortality hours after treatment (h)				
(400 mg kg^{-1})	24	48	72	96	LSD
S. zeamais					
Ethyl acetate	5±0.50	15±0.95	25±1.25	80±2.44	10.40
Chloroform	0±0.00	15±0.95	30±1.73	45±1.25	3.60
Hexane	0±0.00	0±0.00	5±0.50	15±0.50	9.60
Butanol	0±0.00	5±0.50	5±0.50	10±0.81	NS
Aqueous	5±0.50	5±0.50	20±0.81	0±0.00	18.00
C. maculatus					
Ethyl acetate	0±0.00	0±0.00	10±0.81	10±0.81	NS
Chloroform	0±0.00	0±0.00	0±0.00	0±0.00	NS
Hexane	5±0.50	5±0.50	20±0.81	20±0.81	NS
Butanol	0±0.00	5±0.50	15±0.95	15±0.95	NS
Aqueous	5±0.50	5±0.50	10±0.81	10±0.81	NS

Means of four replicates of 10 insects each; LSD test ($p \leq 0.05$); NS = Non-significant

Table 3: Mean mortality (%) of *S. zeamais* and *C. maculatus* on exposure to grains treated with various extract fractions of *D. arborea*

Extract fractions	Mean percent mortality at different times after treatment (h)				
(400 μl mL^{-1})	24	48	72	96	LSD
S. zeamais					
Ethyl acetate	0±0.00	5±0.57	5±0.81	10±1.00	4.75
Chloroform	0±0.00	5±0.57	0±0.50	5±0.05	3.00
Hexane	0±0.00	5±0.81	5±0.50	5±0.50	3.60
Butanol	0±0.00	5±1.00	5±1.00	5±1.00	NS
Aqueous	5±1.15	5±1.151	5±1.291	5±1.47	8.45
C. maculatus					
Ethyl acetate	5±1.41	25±2.44	30±2.98	40±3.68	18.60
Chloroform	10±2.87	30±3.77	35±4.69	45±5.83	29.85
Hexane	10±2.16	35±2.71	40±2.60	60±2.98	12.20
Butanol	10±2.06	30±1.25	35±1.82	50±1.71	11.70
Aqueous	15±1.91	20±2.50	20±2.50	25±2.06	15.20

Means of four replicates of 20 insects each. LSD test ($p \leq 0.05$) NS = Non significant

Chloroform and hexane fractions produced significant mortality of 45 and 15%, respectively against *S. zeamais* after 96 h of insect exposure to treated filter papers. Furthermore, the aqueous fraction induced significant mortality of 60% in *S. zeamais* after 96 h of treatment. However, no significant effect was recorded from the different extract fractions against *C. maculatus*.

Table 4: Mean weight loss (%) caused by *S. zeamais* and *C. maculatus* on grains treated with the various extract fractions of *D. arborea*

Extraction fractions (400 mg kg^{-1})	Mean percent weight loss	
	S. zeamais	*C. maculates*
Ethyl acetate	0.88±0.43	0.43±0.50
Chloroform	0.84±0.44	0.75±0.31
Hexane	0.08±0.67	0.45±0.23
Butanol	0.07±0.20	0.15±0.67
Aqueous	0.79±0.44	0.51±0.54
Control	7.24±1.91	6.88±2.79
LSD	1.31	1.81

LSD test ($p \leq 0.01$)

Table 5: Mean number of *S. zeamais* produced in grains treated with extract fractions of *D. arborea* at different days after oviposition

Extraction fractions	Days after oviposition period		
	1	7	14
S. zeamais			
Ethyl acetate	4.08	5.38	9.46
Chloroform	5.10	7.49	10.01
Hexane	3.25	4.56	8.27
Butanol	3.47	5.21	9.06
Aqueous	2.09	4.31	7.53
Control	5.10	15.0	35.00
LSD	1.31	1.81	1.87
C. maculatus			
Ethyl acetate	1.65	2.89	3.51
Chloroform	1.87	1.98	2.05
Hexane	1.78	2.37	2.95
Butanol	0.32	0.49	0.76
Aqueous	1.41	2.56	3.01
Control	3.00	8.00	20.00
LSD	0.81	1.07	1.51

Means±SEM of four replicates of 20 insects each LSD test ($p \leq 0.01$)

Toxicity of the extract fractions on grains: Insect mortality observed on grains treated with the different extract fractions of *D. arborea* differed with respect to the two insect species (Table 3). *Sitophillus zeamais* was less affected compared to *C. maculatus*. After 96 h of treatment, the aqueous fraction produced 15% mortality of *S. zeamais*, while a mortality of 60% was observed in hexane fraction against *C. maculatus*.

Level of protection offered by the extract fractions: There were significant differences ($p \leq 0.01$) amongst the extract fractions of *D. arborea* in reducing damage caused by the beetles. Minimal weight loss of 0.08 and 0.43% was observed for maize and cowpea, respectively from the hexane and ethyl acetate fractions against *S. zeamais* and *C. maculatus* (Table 4).

Effect on immature stages and progeny production: Maize and cowpea grains treated with the different extract fractions of *D. arborea* significantly ($p \leq 0.01$) affected the immature stages of *S. zeamais* and *C. maculatus* (Table 5). Butanol fraction followed by chloroform fraction recorded significant effect on the immature stages of *C. maculatus*. The extract fractions significantly affected

Table 6: Effect of extract fractions of *D. arborea* on F1 progeny produced by *S. zeamais* and *C. maculatus*

Extraction fractions	Mean number of F1 progeny	
	S. zeamais	*C. maculates*
Ethyl acetate	28.75±3.95	20.50±8.38
Chloroform	27.50±9.95	19.25±5.50
Hexane	21.25±7.63	30.50±10.4
Butanol	21.50±7.114	17.50±5.57
Aqueous	27.00±8.98	20.00±4.39
Control	42.00±5.23	52.00±7.30
LSD	11.04	1.81

Mean of four Replicates of 20 insects each LSD test ($p \leq 0.01$)

Table 7: Repellency of extract fractions of *D. arborea* exerted on *S. zeamais* and *C. maculatus*

Extraction fractions	Percent repellency	
	S. zeamais	*C. maculatus*
Ethyl acetate	44	25
Chloroform	44	6
Hexane	38	42
Butanol	25	13
Aqueous	50	33
Overall PR	40	24
LSD	11.04	17.46

Means of four Replicates of 10 Insects each, LSD test ($p \leq 0.01$)

the F_1 generation produced by *S. zeamais* and *C. maculatus* (Table 6). The butanol fraction inhibited progeny production of the two insect species more than the other fractions. However, the extract fractions were able to reduce the F_1 progeny of *S. zeamais* and *C. maculatus* compared with the untreated control.

Repellency bioassay: The different extract fractions of *D. arborea* showed different levels of repellency to the two insect species (Table 7). *Stiphillus zeamais* was more repelled with an overall repellency value of 40%, while *C. maculatus* was less repelled with an overall repellency value of 24%. Ethyl acetate fraction significantly repelled *S. zeamais* and *C. maculatus* by about 44 and 25%, respectively. However, a more significant effect was observed in the aqueous fraction where *S. zeamais* and *C. maculatus* were repelled by 50 and 33%, respectively.

DISCUSSION

The extract fractions of *D. arborea* applied topically against *C. maculatus* and *S. zeamais* caused significant mortality of the two insect species and this possibly is attributable to the presence of secondary metabolites identified as Mannispirostan A and Spiroconazole A (Okunji *et al*., 1996). *Callosobruchus maculatus* was more susceptible to contact action of the extract fractions impregnated on grains than *S. zeamais* probably because of the absence of hard and highly sclerotized thoracic cuticle as in the latter. The significant mortality of *S. zeamais* in filter paper treated with the extract fractions

indicates possible residual toxic effect of *D. arborea* on this species. The contact and residual actions of *D. arborea* on the two insect species suggests that it possesses some insecticidal properties. Previously, Epidi *et al.* (2008) reported that the leaf powder of *D. arborea* caused significant mortality of these insect species. Also, the significant reduction in damage shows the plant may contain some antifeedant properties (Niber, 1994).

The inhibition of the development of eggs and immature stages within grain kernels suggest the presence of ovicidal properties in the plant (Udo *et al*., 2004). This increases the protectant potential of *D. arborea* against insect damage in storage. Also, the repellent action observed against the two insect species is noteworthy as this would prevent the insect from settling, feeding and laying eggs (Saxena, 1985).

The results obtained from the study suggest good potential for the use of *D. arborea* in storage pest management systems particularly in Africa. *D. arborea* is apparently safe as it already has ethno-botanic uses including treatment of certain ailments (Okunji *et al*., 1996; Etukudo, 2003). This study therefore recommends the use of *D. arborea* extracts in management of *S. zeamais* and *C. maculatus* infesting stored maize and cowpea, respectively.

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