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Brief Communication

Insecticidal and vertebrate toxicity associated with ethnobotanicals used as post-harvest protectants in Ghana

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Abstract

Six plant species (*Cassia sophera*, *Chamaecrista nigricans*, *Mitragyna inermis*, *Ocimum americanum*, *Securidaca longepedunculata* and *Synedrella nodiflora*) traditionally used in Ghana to control insect pests of stored grain and legumes were screened in the laboratory at three concentrations (0.5, 1 and 5%, w/w) against four common storage pests (*Rhyzopertha dominica*, *Callosobruchus maculatus*, *Sitophilus zeamais* and *Prostephanus truncatus*). All the plants showed some ability to control all or some of the test insect species. Levels of efficacy varied according to test concentration with the highest concentration tested providing the best control. The *S. longepedunculata* plant induced the highest percent mortality and was the best at reducing emergence of the F_1 generation. The six plants were also incorporated into standard rat diet at two concentrations (1 and 5%, w/w) and fed to rats over a 6-week period to assess potential deleterious effects against vertebrates. None of the plants demonstrated any neurotoxicological or neurobehavioural effects to the rats over the course of the trial. However, *S. longepedunculata* and *C. nigricans* caused a significant reduction in rat growth rate when incorporated at 5% in the diet, induced cell hyperplasia in the liver, and reduced the mean weight of the liver and kidneys, compared to the control group of rats. Kidney pathology was affected only by the 5% concentration of *S. longepedunculata* which caused a reduced accumulation of $\alpha 2\mu$-globulin. The implications of these results are discussed in the context of farmer usage of insecticidal plants for stored product protection. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Plant materials with insecticidal properties have been used traditionally for generations throughout the world. Botanical treatments are particularly relevant for small-scale subsistence farmers during post-harvest storage of their commodities (Proctor, 1994; Dales, 1996). In this context, botanicals have many advantages over synthetic pesticides because they are normally gathered locally by producers and can provide an inexpensive method of pest control during storage. For the majority of farmers in the world, commercial insecticides are often too costly or unavailable. Similarly, many uneducated producers use synthetic pesticides inappropriately, leading to environmental and human safety hazards (Levine, 1991) as well as promoting insecticide resistance. These factors have led to increased efforts to understand indigenous pest control strategies with a view to reviving and modernising the age-old practices (Poswal and Akpa, 1991; Niber, 1994; Bloszyk et al., 1995; Onu and Aliyu, 1995; Xie et al., 1995; Chimbe and Galley, 1996; Rajapakse and Emden, 1997; Shaaya et al., 1997).

Previous studies assessing the insecticidal properties of plants have often failed to assess simultaneously their safety. Potential vertebrate toxicity associated with the mixing of insecticidal plants with stored food requires investigation before the institutional promotion of insecticidal plants at farm level. The present study aims at identifying plants that are used traditionally by subsistence farmers of Ghana as post-harvest protectants (Cobbinah et al., 1999), and screening them for bioactivity against target pests as well as for potential deleterious effects against vertebrates.

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2. Materials and methods

2.1. Insect toxicity bioassay

Rhyzopertha dominica (Fabricius) (Coleoptera: Bostrichidae) were reared on wheat (*Tritium aestrium*), *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) were reared on cowpea (*Vigna unguiculata*) and *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) and *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) were reared on maize (*Zea mays*). Pre-equilibrated commodity (100 g, 27±5°C, 60±5% relative humidity) was placed in 250-ml glass jars. Roots of *Securidaca longepedunculata* and leaves of the other plant species (Table 1) were shade-dried in Ghana, shipped to the UK and then milled to a fine powder. The plant powder was admixed (w/w) with the commodity at three different concentrations (0.5, 1.0 and 5.0%, plus untreated control). 40 known-age insects were added to each jar with respect to their commodity type, and the jars were placed in a controlled temperature and humidity (CTH) room (27±5°C, 60±5% relative huidity). Parental insects were removed after 7 days, and the treatments were scored at 49 days (28 days for *C. maculatus*) to record the number of live and dead insects in the F_1 emergence.

2.2. Vertebrate toxicity bioassay

Weanling rats were assigned to 14 groups of six rats each (two control groups and 12 experimental groups). Rats were acclimatised on standard rat diet for 2 weeks prior to trial commencement. Plant materials prepared as for the insect bioassays were incorporated into the diet at 1 and 5% (w/w) and fed to rodents over a 6-week period. The parameters measured daily during the trial were dietary intake and growth rate. Rodent behavioural parameters were assessed at trial mid-point and at trial completion only and included: general appearance, grooming, incontinence, alertness, muscle tone, handling response, startle response, righting reflex, gait, gross tremor, twitches, aggression, fearfulness and stereotypy.

Animals were examined by an experienced observer (D.E.R.). The authors have assessed rodent behaviour in similar studies using the known fungal neurotoxin, Penetrem A, and the pyrethroid insecticide, Deltamethrin, both of which have yielded clear positive results. At the end of the trial, rat kidneys and livers were visually and histologically examined.

3. Results

3.1. Insect toxicity bioassay

Securidaca longepedunculata was the most effective treatment for controlling all the tested insects (Table 2, Fig. 1). The observed mortality and decreased F_1 emergence were dose dependent, and percent mortality was as high as 70% in *R. dominica* trials at 5% (w/w). Although *Chamaecrista nigricans* did not directly increase the percent mortality in the F_1 generation, it dose-dependently reduced the overall emergence numbers of the F_1 generation in all the insect species as compared with the untreated control. *Ocimum americanum* dose-dependently increased the mortality of *P. truncatus*, *S. zeamais* and *C. maculatus*, while reducing F_1 emergence in all species. *Cassia sophera* was most effective against *C. maculatus* and *R. dominica* but also reduced F_1 emergence of *S. zeamais* at the 1 and 5% concentrations. *Mitragyna inermis* increased the mortality of *R. dominica* and *C. maculatus* and reduced the emergence of *S. zeamais* and *P. truncatus*. The least effective plant was *Synedrella nodiflora*, which could only increase mortality of *R. dominica* and reduce F_1 emergence of *C. maculatus*.

3.2. Vertebrate toxicity bioassay

Dietary intake and weight gain were normal in all treatments except for *S. longepedunculata* and *C. nigricans* fed at 5% (w/w) (Fig. 2b). However, reduced weight gain was not low enough to necessitate a change in experimental protocol to include pair feeding whereby dietary intake in control groups is matched with treatment groups. None of the treatments, including *S. longepedunculata* and *C. nigricans*, affected weight gain when fed at 1% (w/w) (Fig. 2a).

Table 1
The plants from Ghana that were used in insect and vertebrate toxicity bioassays

Local name	Latin name	Methods of use cited by farmers for post-harvest protection
Tikublaakum	*Cassia sophera* (Leguminosae)	Powdered leaves admixed or layered with commodity
Lodel	*Chamaecrista nigricans* (Leguminosae)	powdered leaves admixed with commodity or placed at base of silo
Dekonja	*Mitragyna inermis* (Rubiaceae)	Whole or powdered seeds or powdered leaves admixed with commodity; commodity immersed 30 s in water from boiled leaves
Kpasiuk	*Ocimum americanum* (Labiatae)	Whole or powdered mature plants admixed or layered with commodity
Palaga	*Securidaca longepedunculata* (Polygalaceae)	Commodity immersed 30 s in water from soaked roots; commodity admixed with powdered roots
Kimkim	*Synedrella nodiflora* (Labiatae)	Commodity immersed 30 s in water from boiled leaves or whole plant; commodity admixed with powdered leaves

Table 2
The effect of six plant materials on the F_1 adult mortality and emergence of four storage insect pests[a]

	F_1 adult mortality (mean% mortality)			F_1 adult emergence (mean live insects)		
Plant admix concentration against insect species	0.5%	1.0%	5.0%	0.5%	1.0%	5.0%
Cassia sophera						
C. maculatus	21.3	22.3	35.6*	101.4*	75.8*	54.3*
P. truncatus	11.3	11.3	10.9	178.2	146.4	136.6
R. dominica	1.6	2.5	8.6*	343.4*	300.0*	137.8*
S. zeamais	2.7	1.9	3.9	177.0	164.0*	136.4*
Chamaecrista nigricans						
C. maculatus	24.0	20.0	20.9	107.6	100.6	80.0*
P. truncatus	9.5	9.3	7.7	142.1	136.4*	112.2*
R. dominica	2.7	2.8	3.6	542.4	446.2*	410.4*
S. zeamais	2.6	1.3	1.1	206.3	169.6*	146.5*
Mitragyna inermis						
C. maculatus	15.0	35.3*	39.8*	134.6	128.5	100.7*
P. truncatus	1.9	0.7	0.7	259.2	209.5*	155.6*
R. dominica	1.5	8.9*	14.0*	345.6	322.2	191.3*
S. zeamais	2.4	4.2	19.6*	196.1	175.4*	121.0*
Ocimum americanum						
C. maculatus	15.0	45.3*	64.1*	120.4	44.3*	43.8*
P. truncatus	5.0*	9.4*	18.2*	339.2	241.2*	240.5*
R. dominica	3.0	2.8	6.7*	318.0	219.3*	199.0*
S. zeamais	4.9*	15.3*	19.5*	262.2	151.6*	138.1*
Securidaca longepedunculata						
C. maculatus	18.3	22.0	25.1*	131.8	89.0*	84.2*
P. truncatus	15.5*	26.5*	38.1*	70.6*	56.6*	47.0*
R. dominica	29.3*	59.2*	75.4*	86.0*	30.6*	9.4*
S. zeamais	7.9*	22.6*	34.1*	106.4*	74.6*	52.4*
Synedrella nodiflora						
C. maculatus	15.6	22.1	18.3	41.2*	40.4*	45.7*
P. truncatus	2.8	3.1	3.4	218.4	200.5	201.4
R. dominica	2.4*	4.9*	6.8*	451.3	445.5	439.0
S. zeamais	1.2	1.4	1.8	179.0	176.8	145.8

[a] Treatments marked with * are significantly different from the control (Mann–Whitney U-tests, $n=5$, $P<0.05$).

All rats were considered to exhibit normal behaviour in all treatments at trial mid-point and at the end of the 6 weeks. On post-mortem examination, livers and kidneys appeared normal, with the exception of the 5% concentration of *S. longepedunculata,* where kidneys were pale and small. The weights of livers and kidneys were normal with the exception of the 5% concentrations of *S. longepedunculata* and *C. nigricans* (Table 3). Because the initial weights of rats in the 1% treatments of *C. sophera* and *M. inermis* were slightly lower than the control group, their organ weights appear significantly different despite no differences in cumulative growth rate for these two groups. Kidney pathology showed no significant findings, with the exception of the 5% concentration of *S. longepedunculata*, where reduced accumulation of α2μ-globulin was observed. This protein is a male-specific urinary constituent in rats. It has been implicated in nephrotoxicities in male rats resulting from exposure to a diverse range of light hydrocarbons by non-covalent binding with the xenobiotic. However, these toxicities are typified by the accumulation of hyaline droplets (Hard et al., 1993), and this accumulation was not observed in the present study. Liver pathology showed increased mitotic division in the 5% concentrations of *S. longepedunculata* and *C. nigricans*. Hyperplasia in the liver was due to increased cell death, and all 5% treatments and some 1% treatments showed slightly increased rates of mitosis. Increased DNA replication can provide a cellular environment for mutation and so an induction of this response merits further investigation.

4. Discussion

All of the plant materials showed some ability to control storage pests, confirming their ethnobotanical uses. As expected, the efficacy of the plant materials to control storage pests varied among the insect and plant species. The concentration used was important, and many of the plants showed classical dose-dependent effects that occur with conventional insecticides. The

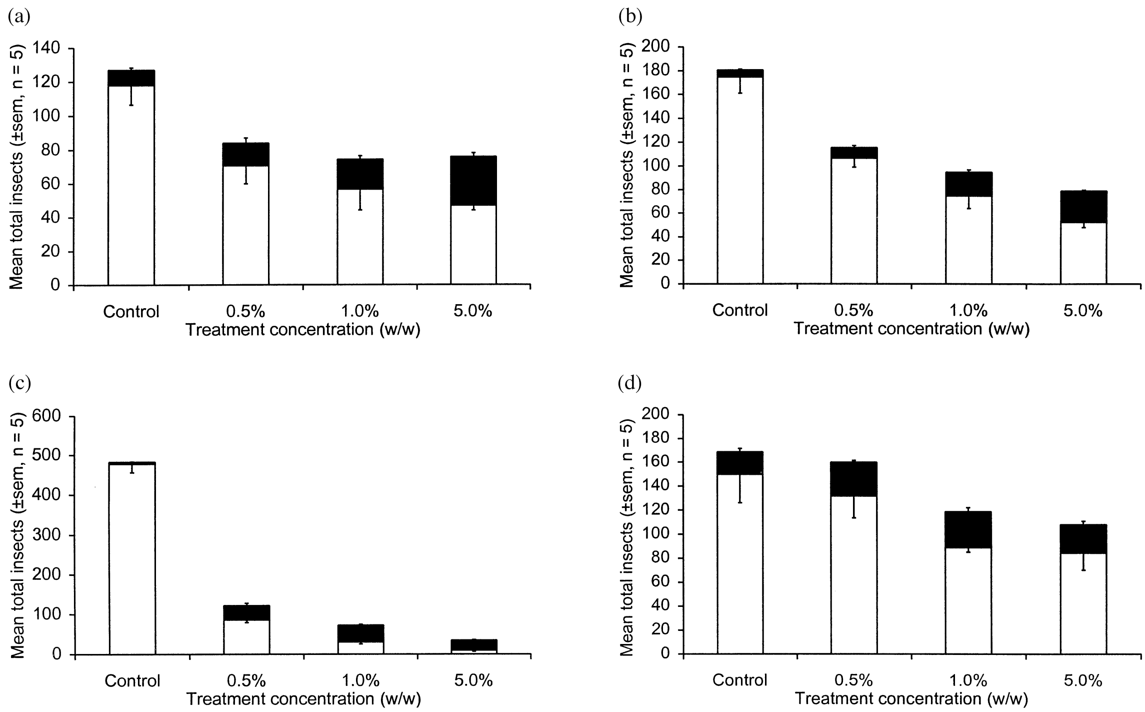

Fig. 1. The effect of Securidaca longepedunculata on F_1 adult emergence and mortality of (a) *Prostephanus truncatus*; (b) *Sitophilus zeamais*; (c) *Rhyzopertha dominica*; (d) *Callosobruchus maculates*.

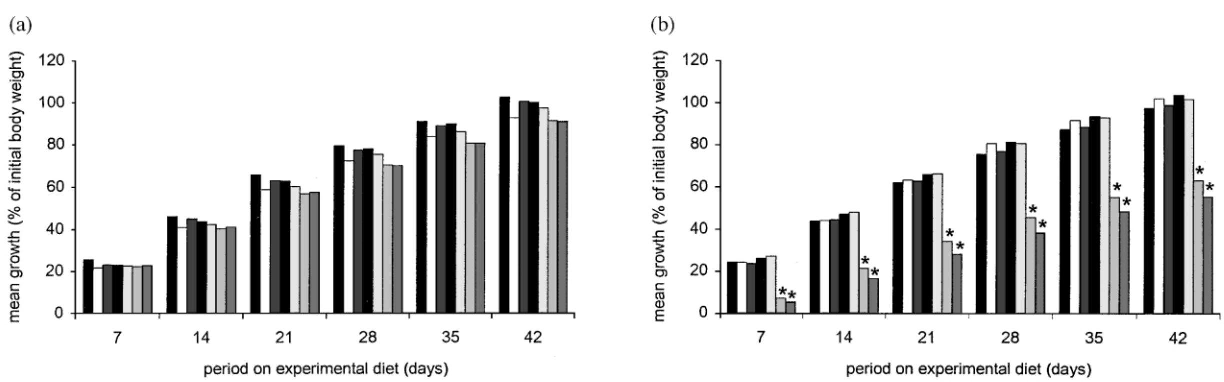

Fig. 2. The effect of six plant materials on rodent growth rate when incorporated into their diet at: (a) 1% (w/w); (b) 5% (w/w). Bars marked with * are significantly different from the control (Mann–Whitney U-test, $n=6$, $P<0.05$).

Table 3
The mean weight of kidneys (weighed as a pair) and liver from rats fed one of six insecticidal plant materials at two different concentrations in their diet[a]

	Kidney weight (g±S.E.M.)		Liver weight (g±S.E.M.)	
Plant material	1% (w/w)	5% (w/w)	1% (w/w)	5% (w/w)
Control	1.82±0.039	1.79±0.041	11.7±0.18	11.1±0.18
C. nigricans	1.75±0.042	1.58±0.025*	10.5±0.27	8.2±0.27*
C. sophera	1.61±0.070*	1.77±0.075	9.9±0.50*	11.0±0.47
M. inermis	1.52±0.076*	1.77±0.018	8.8±0.44*	10.8±0.09
O. americanum	1.73±0.023	1.76±0.065	10.7±0.49	10.8±0.46
S. longepedunculata	1.77±0.045	1.53±0.045*	10.9±0.28	9.2±0.19*
S. nodiflora	1.71±0.038	1.71±0.035	10.5±0.28	11.0±0.15

[a] Values marked with * are significantly different from the control (Mann–Whitney U tests, $n=6$, $P<0.05$).

observed variability among insect species' susceptibilities is a common biocidal phenomenon. However, as phytochemical information about these plants is unavailable, it is not known how variability of secondary metabolites has biased our results. Some of the plants did not cause F_1 adult mortality although they did decrease overall emergence of the F_1 generation. Further research to establish precise mode(s) of action is ongoing.

The most effective plant materials identified from these experiments for controlling storage insect pests, *S. longepedunculata* and *C. nigricans*, were also the most potentially toxic to vertebrates on ingestion. This was demonstrated by reduced weight gain and changes to the rat kidney and liver when fed at the higher concentration of 5% (w/w). However, none of the rats fed with these two plants at this level exhibited any obvious behavioural changes over the 6-week experimental period. Given the absence of any signs of neurological or neurobehavioural disturbance over this period, even at a dose level sufficient to cause reduced weight gain, it was not considered necessary to carry out any other neurotoxicological studies. Potential chronic toxicity associated with these plants will need to be evaluated through long-term bioassays.

In conclusion, it can be reliably assumed that farmers will not be ingesting these plant materials in concentrations as high as 5% (w/w). Farmers who use plant materials during on-farm storage of their commodities remove most of the plant material before consumption through washing and winnowing. However, it is not known how much plant material residue is potentially consumed. Medicinal uses of *S. longepedunculata* have been reported from other parts of Africa (Tommasi et al., 1993; Kassa et al., 1998; Olajide et al., 1998), providing some information that the plant should be relatively safe to use if only small quantities are ingested. Future research should attempt to measure potential residues left on plant-treated grain after storage and determine whether there are any likely chronic toxicity effects from these residues.

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