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Ethnobotanicals - Future prospects as post-harvest insecticides

INTRODUCTION

Plants are well-known for their medicinal and insecticidal properties, and considerable research around the world is conducted to screen plants for new drugs and agrochemicals. Because of the overwhelming diversity of plant species and costs involved in developing new drugs or chemicals, indigenous knowledge systems have often been employed to develop new leads on ethnobotanicals. Many countries require incentives to preserve their biodiversity and, since the UN Convention on Biological Diversity (CBD) in 1992, developing countries have had the means to implement appropriate legislation to protect indigenous knowledge. The process of scientifically validating ethnobotanical information will add value to a State's biological resources and, in conjunction with the CBD, promote sustainable ecosystem management.

Plant materials with insecticidal properties have been used for generations throughout Africa, Asia and the Americas. Botanical treatments are particularly relevant for small-scale subsistence farmers during post-harvest storage of their commodities (1). In this context, botanicals have many advantages over synthetic pesticides because they are normally gathered locally by farmers 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 are unavailable. Also, many uneducated farmers use synthetic pesticides inappropriately, leading to environmental and human safety hazards as well as promoting insecticide resistance. These factors have led to increased efforts to understand indigenous pest control strategies (2,3) with a view to reviving and modernising age-old practices.

The objectives of our research have been to identify plants from Ghana that are traditionally used by subsistence farmers as post-harvest protectants (4), screen them for bioactivity through various target organism mode-of-action bioassays, identify the relevant phytochemicals responsible for activity and establish vertebrate toxicity associated with botanicals as a potentially residual food additive. This information is being used to optimise and promote the usage of botanicals by small-scale farmers in Africa. However, the discovery of new modes of action could lead to new commercial products, providing wider economic benefits.

Table I - Plants from Ghana that were involved in toxicity or repellency bioassays

Local name	Latin name	Methods of use cited by farmers for post-harvest protection
Neem	*Azadirachta indica*	seed oil, powder, paste or water extract, fresh or dried whole leaves, leaf powder, paste or water extract, admixed or layered
Chilli pepper	*Capsicum annuum*	crushed or whole fruit, admixed or layered
Lodel	*Chamaecrista kirkii*	powdered leaves, admixed or placed at base
Tikublaakum	*Cissus populnea*	powdered leaves
Orange peel	*Citrus sinensis*	admixed powdered peel
Famitatagba	*Combretum* sp.	water from boiled leaves, immersed 20-30 sec., admixed powdered leaves
Youlaga	*Grewia mollis*	admixed ash from branches or powdered leaves,
Kuga	*Khaya senegalensis*	admixed powdered bark or leaves,
Lidikonja	*Lippia multiflora*	whole leaves and/or flowers, dried and layered
Dekonja	*Mitragyna inermis*	whole or powdered seeds, water from boiled leaves, powdered leaves
Kpasiuk	*Ocimum americanum*	whole or powdered mature plants, admixed or layered
Kanbam	*Pleiocapa mutica*	admixed powdered roots bark and/or leaves
Nae	*Pterocarpus erinaceus*	water extract of leaves and/or roots, admixed powdered leaves
Palaga	*Securidaca longipedunculata*	water from soaked roots, admixed powdered roots
Kimkim	*Synedrella nodiflora*	water from boiled leaves or whole plant, poured or immersed 20-30 sec., powdered leaves
Kulenka	unknown (Gramineae)	whole flower heads, mixed
Shea nut	*Vitellaria paradoxa*	oil or residue from seeds, waste water from seed processing

MATERIALS AND METHODS

Toxicity Bioassay

Pre-equilibrated commodity (100 g, 27 ± 5°C, 60 ± 5% rh) was placed in 250 ml glass jars. Dried and pre-ground plant material, identified during surveys in Ghana (Table I), was admixed (w/w) with the commodity at three different concentrations (0.5%, 1.0% and 5.0%, plus untreated control). Forty known-age insects were added to each jar containing the relevant commodity type. *Rhyzopertha dominica* were reared on wheat (*Tritium aestrium*), *Callosobruchus maculatus* were reared on cowpea (*Vigna unguiculata*), and *Sitophilus zeamais* and *Prostephanus truncatus* were reared on maize (*Zea mays*). The jars were placed in a controlled temperature and humidity (CTH) room (27 ± 5°C, 60 ± 5%rh) and were scored at 7 days (1 day for *C. maculatus*) and at 49 days (28 days for *C. maculatus*) to record the number of live and dead insects.

Repellency Bioassay

Repellency of a plant material to an insect was assessed using a choice preference arena. The arena consisted of a plastic box (323 mm x 323 mm x 158 mm, Stewart Plastics Ltd.) with a cardboard sheet taped on the inside base. Two 50 g piles of pre-equilibrated commodity (27 ± 5°C, 60 ± 5% rh) were placed in the box in opposite corners to each other. One pile had been previously treated with ground plant material (5% admix, w/w), whereas the other pile was left untreated. Two untreated piles were used as a 'no-choice' control replicate. Forty known-age insects were placed in the centre of the arena between the two piles. The number of insects in each pile was scored 24 hours after the insects were added. Replicates were placed concentrically in a CTH room (27 ± 5°C, 60 ± 5% rh) to account for potential phototactic orientation.

RESULTS

Toxicity Bioassay

Results showed that *Securidaca longipedunculata* (palaga) was the most effective treatment for controlling the tested insects (Table II, Figure 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 (Figure 1c). Although *Chamaecrista kirkii* (lodel) did not directly increase the percent mortality found in the F_1 generation, it dose-dependently reduced the overall emergence numbers of the F_1 generation in all the insect species when compared with the untreated control. *Pleiocapa mutica* (kanbam) had a similar effect to *C. kirkii* against *R. dominica, C. maculatus* and *S. zeamais*. *Grewia mollis* (youlaga) was shown to be effective by increasing mortality and decreasing emergence against *R. dominica* and *S. zeamais*. *Ocimum americanum* (kpasuik) dose-dependently increased mortality of *P. truncatus, S. zeamais* and *C. maculatus*. *Mitragyna inermis* (dekonja) increased mortality of *R. dominica* and *C. maculatus* and reduced emergence of *S. zeamais* and *P. truncatus*. *Azadirachta indica* (neem) reduced emergence of *R. dominica, S. zeamais* and *P. truncatus* by as much as 50%. *Capsicum annuum* (chilli pepper) reduced emergence of *C. maculatus, S. zeamais* and *R. dominica*. Similar results to *C. annuum* were obtained with *Khaya senegalensis* (kuga) for all the insects.

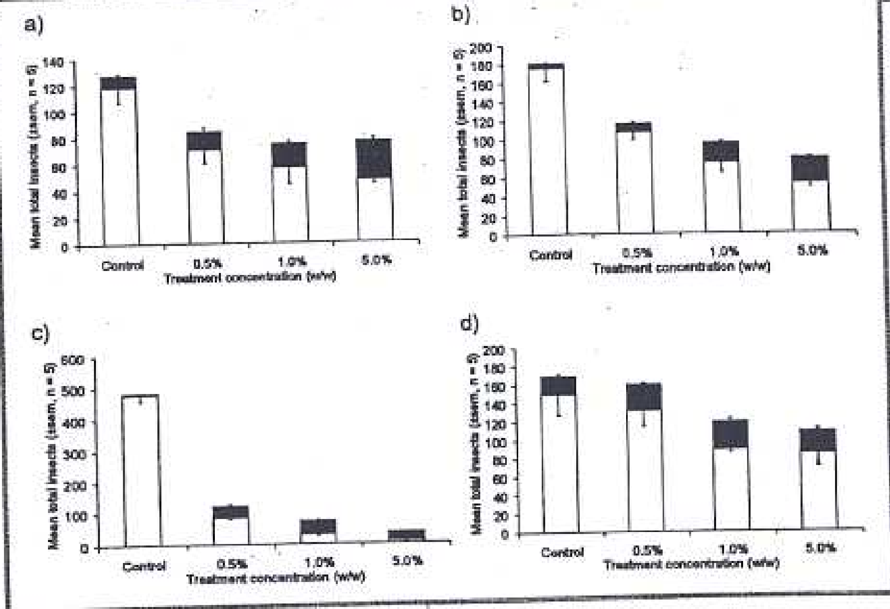
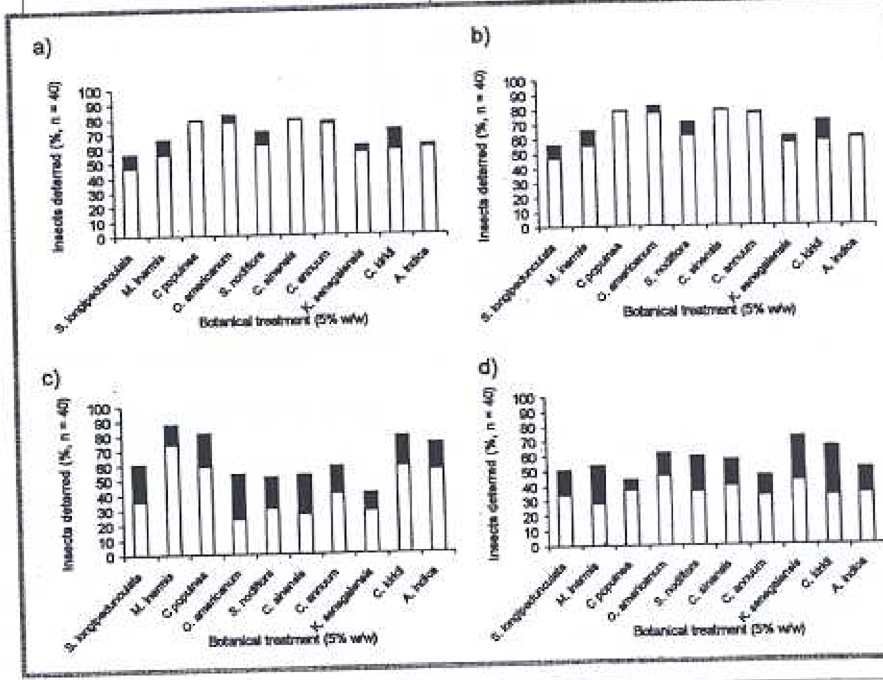


Figure 1 - Effect of *Securidaca longipedunculata* upon F_1 emergence of a) *Prostephanus truncatus*; b) *Sitophilus zeamais*; c) *Rhyzopertha dominica*; d) *Callosobruchus maculatus*
■ Dead insects; □ Live insects

Repellency Bioassay

Results showed that *P. truncatus* were the most repelled by plant-treated commodity, followed by *R. dominica, C. maculatus*, and *S. zeamais* (Figure 2, Kruskal-Wallis among insects, $\chi^2 = 8.3$, df = 3, $P < 0.05$, Mann-Whitney U-tests between paired species $Z > 2.5$, n = 11, $P < 0.01$). Plant materials were considered to be deterrent when significantly greater than 50% of the insects were found either in the untreated pile or elsewhere in the container (Spearman correlation between neither and untreated = 0.791, $P < 0.01$; between neither and treated = 0.108, $P > 0.05$).

Figure 2 - Deterrency (%) of different botanicals admixed with commodity against a) *Prostephanus truncatus*; b) *Sitophilus zeamais*; c) *Rhyzopertha dominica*; d) *Callosobruchus maculatus*



CONCLUSIONS

Existing scientific knowledge on the plants that have been identified and tested varies considerably. *Azadirachta indica* is perhaps the most widely referred-to botanical (3000+ references via CAB Abstracts); however, some other plant species identified are similarly cosmopolitan in their geographic occurrence and traditional uses as medicines or insecticides [*Capsicum annuum* (600+), *Citrus sinensis* (300+), *Khaya senegalensis* (100+), *Ocimum americanum* (100+)]. Little phytochemical or mode of action research has been published on the other post-harvest botanicals identified. Other laboratory studies have confirmed the insecticidal activity of a small number of Ghanaian plant materials traditionally used as storage protectants (5,6).

As expected, the efficacy of plant materials varied among the insect and plant species. The concentration was important, and many of the plants showed classical dose-dependent effects that occur with conventional insecticides. The observed variability among insect species'

Table II - Analysis of bioassays using Mann-Whitney U-tests
Values marked with * are significantly different from the control, n = 5, P < 0.05

Plant admix concentration against insect species		F1 adult mortality			F1 adult emergence		
		0.5%	1.0%	5.0%	0.5%	1.0%	5.0%
Azadirachta indica	*C. maculatus*		*				*
	P. truncatus		*	*			*
	R. dominica	*	*	*	*	*	*
	S. zeamais					*	*
Capsicum annuum	*C. maculatus*			*	*	*	*
	P. truncatus						*
	R. dominica	*	*	*	*	*	*
	S. zeamais			*		*	*
Chamaecrista kirkii	*C. maculatus*					*	*
	P. truncatus					*	*
	R. dominica					*	*
	S. zeamais				*	*	*
Cissus populnea	*C. maculatus*			*	*	*	*
	P. truncatus						
	R. dominica			*	*	*	*
	S. zeamais					*	*
Citrus sinensis	*C. maculatus*						
	P. truncatus						
	R. dominica			*	*	*	*
	S. zeamais					*	*
Combretum sp.	*C. maculatus*						
	P. truncatus						
	R. dominica				*	*	*
	S. zeamais						
Grewia mollis	*C. maculatus*						
	P. truncatus						
	R. dominica	*	*	*	*	*	*
	S. zeamais			*	*	*	*
Khaya senegalensis	*C. maculatus*			*		*	*
	P. truncatus						*
	R. dominica		*	*	*	*	*
	S. zeamais					*	*
Mitragyna inermis	*C. maculatus*		*	*			*
	P. truncatus					*	*
	R. dominica		*	*			*
	S. zeamais			*		*	*
Ocimum americanum	*C. maculatus*		*	*		*	*
	P. truncatus	*	*	*		*	*
	R. dominica			*		*	*
	S. zeamais	*	*	*		*	*
Pleiocapa mutica	*C. maculatus*					*	*
	P. truncatus						
	R. dominica				*	*	*
	S. zeamais						*
Securidaca longipedunculata	*C. maculatus*			*		*	*
	P. truncatus	*	*	*	*	*	*
	R. dominica	*	*	*	*	*	*
	S. zeamais	*	*	*	*	*	*
Synedrella nodiflora	*C. maculatus*				*	*	*
	P. truncatus						
	R. dominica	*	*	*			
	S. zeamais						

susceptibilities is a common biocidal phenomenon. Some of the plants did not demonstrate increased percent mortality although they did decrease overall emergence of the F_1 generation. Further research to establish precise mode(s) of action is ongoing.

The standardised methods used in this trial did not always reflect current farmer practice. For example, some of the plants are normally applied as hot water extracts, i.e. kimkim, famitatagba and dekonja. It is these plants, in particular, that did not perform well in these toxicity trials. Therefore, the potential toxicity associated with these plants may only become apparent with differing application methods. Further bioassays as well as phytochemical analysis will help confirm these observations.

The most effective plant material identified from these experiments, *Securidaca longipedunculata*, was not the most widely-used plant by farmers as assessed from the ethnobotanical surveys conducted in Ghana. Its limited use may be a function of limited knowledge about the plant by farmers, or it may be due to valid onstraints to its use, e.g. resource availability or ease of use (7). Research on vertebrate toxicity indicated a very low toxic effect on ingestion during short-term rodent feeding trials (8), but potential long-term chronic effects remain to be tested. Medicinal uses of *S. longipedunculata* have been reported from other parts of Africa (9-11), providing some information that the plant should be relatively safe to use.

Many of the plants identified from the ethnobotanical surveys provided some level of pest control in laboratory trials. However, there are still many questions which remain to be answered before optimal protocols can be developed for their widespread use by subsistence farmers. To increase the quality and reliability of pest control when using these botanicals, further research is required using field trials and farmer participatory trials under local conditions in Ghana.

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REFERENCES

1. M.J. Dales; A review of plant materials used for controlling insect pests of stored products; Natural Resources Institute, Chatham Maritime, UK, 1996
2. B.T. Niber; *J. of Stored Prod. Res.* **30** 287-301 (1994)
3. D.L. Proctor; Grain storage techniques. Evolution and trends in developing countries; FAO Agricultural Services Bulletin, 1994
4. J.R. Cobbinah, C. Moss, P. Golob, S.R. Belmain; Conducting Ethnobotanical Surveys: An Example From Ghana on Plants Used for the Protection of Stored Cereals and Pulses; Natural Resources Institute, Chatham Maritime, UK, 1999
5. J. R. Cobbinah, G. K. Tuani; *J. Univ. of Sci. & Tech.* **12** 136-139 (1992)
6. G.K. Tuani, J.R. Cobbinah, P.K. Agbodaze; *Ghana J. For.* **1** 43-47 (1994)
7. E. Shaaya, M. Kostjukovski, J. Eilberg; *J. of Stored Prod. Res.* **33** 7-15 (1997)
8. S.R. Belmain, D.E. Neal; in preparation
9. M. Kassa, R. Mshana, A. Regassa, G. Assefa; *Ethiopian J. of Science* **21** 81-89 (1998)
10. O.A. Olajide, S.O. Awe, J.M. Makinde; *Fitoterapia* **69** 245-248 (1998)
11. N. de Tommasi, S. Piacente, F. de Simone, C. Pizza, N. de Tommasi, F. de Simone; *J. of Nat. Prod.* **56** 134-137 (1993)