NATURAL RESOURCES INSTITUTE

Use of Plant Materials for Protecting Farm-Stored Grain against Insect Infestation

Natural Resources Institute

DFID Department for International Development

USE OF PLANT MATERIALS FOR PROTECTING FARM-STORED GRAIN AGAINST INSECT INFESTATION

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CROP POST-HARVEST PROGRAMME

FINAL TECHNICAL REPORT

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Crops Research Institute, Ghana (CRI)
Royal Botanic Gardens, Kew, UK (RBGKew)
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Executive Summary

This research was funded by the Department for International Development's Crop Post-Harvest Programme.

The objective of the research was to investigate local plants in Ghana that are used by farmers to control storage pests as a means of providing cost-effective and environmentally sustainable alternatives to synthetic chemicals.

The major activities conducted were:

- Participatory rural appraisal survey in three Regions of Ghana (Northern, Upper East and Upper West) to determine current farmer preferences and their criteria for selecting plants for use as storage protectants
- Identification and selection of plants used by farmers for involvement in the project
- Screening of candidate plants through laboratory and field trials to assess their efficacy in controlling common storage pests in different stored commodities using different methods of application
- Vertebrate toxicity of plant materials assessed through limited rodent feeding trials
- On-farm trials using selected plant materials conducted with farmers using their storage structures and commodity to gain farmer feedback and to assess the level of control by the plants when used by farmers

Seventy-four percent of villages visited during the PRA survey showed some usage of plant materials as storage protectants. However, the usage of plant materials was geographically biased towards the Upper East Region where the overall number of farmers using plant materials was highest. The usage of plant materials appears to be ethnically and culturally influenced, constraining the availability of indigenous knowledge systems.

The survey showed that all farmers favourably rated the use of plant materials in comparison to synthetics when assessed against the marker criteria of: cost, effectiveness, availability, toxicity, ease of use, acceptability, and versatility.

Seventeen different plant species are currently used by farmers as storage protectants, eight of which are commonly used throughout the northern areas of Ghana. The farmer assessments of marker criteria for each plant species showed that some plant materials were preferred over others, either because of their availability or their ease of use, e.g. less pounding or processing required.

Laboratory and field trials showed that the most effective plant material in controlling storage insects was *Securidaca longipedunculata*. Some of the other plant materials tested showed toxicity (*Chamaecrista kirkii*, *Azadirachta indica*, *Khaya senegalensis*, *Capsicum annuum* *Ocimum americanum*, *Grewia mollis*, *Pterocarpus erinaceus*, *Mitragyna inermis*, *Pleiocapa mutica*) or repellency (*Cissus populnea*, *Citrus sinensis*, *Securidaca longipedunculata*, *Chamaecrista kirkii*, *Khaya senegalensis*, *Mitragyna inermis*, *Azadirachta indica*, *Capsicum annuum*) against some or all of the insect species tested. Control levels were influenced by the method of application and concentration used. Dipping commodity into a hot water extract appeared to be more effective than admixing powdered plant material.

Vertebrate toxicity was negligible when plant materials were incorporated into rodent diets over a six-week period. However, two plant materials, *Securidaca longipedunculata* and

Chamaecrista kirkii, gave some indication they could be harmful if ingested at high concentrations over prolonged periods of time.

Farmer trials are ongoing; their final assessment will occur as part of the next phase of the project at the end of the storage season during May 1999. Initial results are showing variable efficacy among different treatments. These on-farm results will allow recommendations to be formulated on which plant materials could be used at farm level and/or require further research.

Introduction

Post-harvest losses of stored grain have been highlighted by the World Food Summit as a serious constraint to the alleviation of poverty in developing countries. Farmers face considerable storage problems as a result of infestation by insect pests. These must be overcome if the farmer is to maximise his or her income from production of durable food crops. In the past, insect infestation was not a problem of consequence because farmers cultivated traditional varieties which, although low-yielding, were insect resistant. However, the introduction of high-yielding grain and pulse varieties has resulted in increased storage losses as these varieties are usually more susceptible to insect damage. For example, DFID-funded research on the Larger Grain Borer (LGB) in Ghana has shown that shelled maize and maize on the cob can suffer 9% and 20% weight loss, respectively, after only five months storage when LGB is present. When high-yielding varieties of maize are stored (e.g. improved self-pollinating composites), losses have been found to be in excess of 25% after six months in store, even in the absence of LGB.

Market liberalisation in Africa has resulted in more commodities being stored in small quantities in on-farm facilities. This has resulted in increased post-harvest losses, especially by storage pest insects of cereals and legumes. The use of pesticides in food stores at the farm level can be considered costly by resource-poor farmers. Often farmers lack the financial resources to buy good quality commercial insecticides, and when used inappropriately, pesticides can result in risks to human and environmental health and cause insect resistance. Perhaps because of the inappropriate use of pesticides, many farmers have expressed concern over the safety of commercial pesticides; farmers often say they would only use pesticides to treat grain they intend to sell and not to treat grain meant for home consumption. These long-term issues are difficult to resolve quickly, encompassing aspects of grain and pesticide marketing and distribution systems, on-farm storage structure design, and farmer education and literacy levels.

Although synthetic pesticides can work well, their constraints regarding human and environmental safety and insect resistance have led to increased international regulation that reduces the number of 'safe' pesticides available for use. One solution to these problems is to introduce more sustainable methods of pest management through low-cost techniques that are more in tune with the needs of both the population and the environment.

Insecticides of botanical origin have been used for generations as protectants of food against insect infestation. Plant materials have advantages over conventional insecticides in several ways. They are low-cost, are locally and readily available, and do not require any packaging. Dusts obtained from dried powdered plant parts or even simple liquid extracts often contain many toxic chemical constituents which can reduce the development of insect resistance.

Although there has been increasing world-wide scientific research to investigate insecticidal and medicinal uses of plants, much of this research is agrochemically and pharmaceutically derived bio-prospecting research that leads to the identification of novel compounds for new drugs and new synthetic pesticides. During the 1970s, the Natural Resources Institute began to investigate plants as a means of identifying cheap, locally available alternative ways of protecting stored grain as opposed to those relying on the use of conventional insecticides. Information was accrued from both the literature, of which there was very little, and by a global survey. The survey information obtained was published in a descriptive bibliography (Golob & Webley, 1980). This research led to the publication of a database (Rees *et al.*, 1992) and a bulletin (Dales, 1996) on stored product protection by plant materials. Ethnobotanically derived information has led to further bio-prospecting and 'high-potential' research; however, little research has been conducted on improving or optimising current traditional methods and verifying the efficacy of botanicals as used by farmers to protect their stored commodity (Niber, 1994; Xie *et al.*, 1995).

DFID-funded research in Ghana has shown that 7% of farmers surveyed in the Ashanti Region used plant materials as their only method of storage pest control, whereas 25% of farmers used botanicals for storage in combination with other protection methods, i.e. smoke, ash, synthetics (Cobbinah *et al.*, 1999). Surveys in the Northern and Volta regions of Ghana have indicated that plants are used as storage protectants by a small number of farmers. However, most farmers were unaware of the potential of botanicals and do nothing to protect their grain because synthetics are often unavailable or too costly, and these farmers often experience high storage losses. These surveys have shown that there is no consistent method of application and that farmers used botanicals because they were cheap, available and thought to be safer than conventional pesticides. Evidence from the field surveys in Ghana suggests that the poor understanding of the mode of action of botanical materials is preventing the wide-spread uptake of botanical alternatives to synthetic pesticides. For example, DFID-funded research in Ghana has shown that the plant *Chromolaena odorata* (siam weed) is sometimes mixed with stored grain to control storage pests such as *Prostephanus truncatus* and *Sitophilus zeamais*. However, the opinion of farmers with regard to the quality of pest control using *C. odorata* can vary considerably. Whether this difference of opinion among farmers is related to variability in the toxic constituents (Wollenweber *et al.*, 1995; Biller *et al.*, 1994) of *C. odorata* (in different regions, seasons, soil types, etc.), the mode-of-action of the plant (repellency, toxicity, ovicidal, etc.), differences among insect species responses or some other reason (social practice, resource availability, method of use and application), remains unknown.

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COBBINAH, J.R., MOSS, C., GOLOB, P. AND BELMAIN, S.R. (1999) Conducting Ethnobotanical Surveys: an example from Ghana on plants used for the protection of stored cereals and pulses. *NRI bulletin #77*. Chatham, UK: Natural Resources Institute.

DALES, M.J. (1996) A review of plant materials used for controlling insect pests of stored products. *NRI bulletin #65*. Chatham, UK: Natural Resources Institute.

GOLOB, P. AND WEBLEY, D.J. (1980) The use of plants and minerals as traditional protectants of stored products. *TPI report G138*. Chatham, UK: Natural Resources Institute.

NIBER, B.T. (1994) The ability of powders and slurries from ten plant species to protect stored grain from attack by *Prostephanus truncatus* Horn (Coleoptera: Bostrichidae) and *Sitophilus oryzae* L. (Coleoptera: Curculionidae). *Journal of Stored Product Research*. 30(4):287-301.

REES, D.P., DALES, M.J. AND GOLOB, P. (1992) Alternative methods for control of stored product pests: a bibliographic database. *NRI report*, 151 p. Chatham, UK: Natural Resources Institute.

WOLLENWEBER, E., DORR, M. AND MUNIAPPAN, R. (1995) Exudate flavonoids in a tropical weed, *Chromolaena odorata* (L.) *Biochemical Systematics and Ecology*. 23(7/8):873-874.

XIE, Y.S., FIELD, P.G. AND ISMAN, M.B. (1995) Repellency and toxicity of azadirachtin and neem concentrates to three stored-product beetles. *Journal of Economic Entomology* 88(4):1024-1031.

Project Purpose

Effective and sustainable grain management systems developed and promoted.

Specifically, the project objective was to develop cost-effective and safe storage protectants of botanical origin that could offer good control of storage insect pests. The identification and efficacy screening of plant materials already used by farmers during storage is the first step towards promoting the increased usage of insecticidal plants as alternatives to synthetic pesticides.

Research Activities

Participatory rural appraisal survey

The PRA survey showed that a variety of protection methods against insect attack were used by farmers throughout the three northern-most regions of Ghana (Upper East, Upper West and Northern Regions). Out of a total of 32 different methods identified, 19 were using plant materials (8 methods were inert materials such as sand or ash, and 5 methods were synthetic materials). Protection methods were strongly influenced by tribal customs. Gender and commodity type did not appear to influence what type of protection method was used.

Seventy-four percent of villages visited during the PRA survey showed some usage of plant materials as storage protectants. However, usage was geographically biased towards the Upper East Region where the overall number of farmers using plant materials was highest. This is mainly because of tribe-associated indigenous knowledge systems, but wealth was also a factor as poorer farmers were more likely to choose plant materials over synthetics.

The survey showed that all farmers favourably rated the use of plant materials in comparison to synthetics when assessed against the marker criteria of: cost, effectiveness, availability, toxicity, ease of use, acceptability and versatility.

From the 19 methods identified using plant materials, it was found that there were 17 different plant species. The most commonly used (in order) were: *Synedrella nodiflora*, *Capsicum annuum*, *Chamaecrista kirkii*, *Azadirachta indica*, *Securidaca longipedunculata*, Kulenka (unknown species from the Gramineae), *Vitellaria paradoxa*, and *Khaya senegalensis*. The farmer assessments of marker criteria for each plant species showed that some plant materials were preferred over others, either because of their availability or their ease of use. For example, orange peel was thought to work well, but because oranges are grown in the south of Ghana, their use as storage protectants in the north of Ghana is limited Similarly, plant materials that required less pounding or processing before use were more favourably noted.

Complete results of the survey can be found in the report: BRICE, J., MOSS, C., MARSLAND, N., STEVENSON, S., FUSEINI, H., BEDIAKO, J., GBETROE, H., YEBOAH, R. and AYUBA, I. (1996) Post-harvest constraints and opportunities in cereal and legume production systems in Northern Ghana. 3 July – 5 August 1996. Projects R6501, R6502, R6503. Chatham, UK: Natural Resources Institute.

Plants identified and phytochemical information reviewed

In collaboration with the Royal Botanic Gardens, Kew, plant materials were taxonomically identified (Table 1). Due to the nature of the plants, kulenka and famitatagba require further sampling to speciate.

Table 1 Plants from Northern Ghana that are traditionally used as storage protectants

Local name	Latin name	Methods of use cited by farmers
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
Cissus	*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
Dakpezungwari	*Ocimum americanum* var *americanum*	whole or powdered mature plants, water from boiled leaves
Kpasiuk	*Ocimum americanum* var *ocimum*	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

With the exception of *Azadirachta indica*, phytochemical information on most of these plants is lacking. There has been considerable research conducted on *Azadirachta indica* (400+ references), and moderate relevant research on *Capsicum annuum*, *Citrus sinensis* and *Khaya senegalensis* (10 - 50 references). These plants are widely known for their various insecticidal and medicinal properties. Relevant research on other species in Table 1 is low or absent.

Optimum application methods developed through lab and station trials

Laboratory trials

Introduction

Confirming the insecticidal activity of the plants identified from the PRA survey was the first step towards optimising the use of plant materials as storage protectants. The objectives of laboratory trials were to determine which plants were the most effective in killing common storage pests to reduce overall infestation, to determine if application concentration affected efficacy levels and to establish whether the plants could act through other mechanisms such as repellency to prevent infestation during storage.

Materials & methods

Preparation of known-age insects

Insects for the trials (*Callosobruchus maculatus*, *Rhyzopertha dominica*, *Sitophilus zeamais* and *Prostephanus truncatus*) were cultured in three litre glass jars in a controlled environment

(CTH room at 27±5°C, 60±5%rh, 12:12 light:dark). *S. zeamais* and *P. truncatus* were reared on maize (*Zea mays*), *R. dominica* were reared on wheat (*Tritium aestrium*) and *C. maculatus* were reared on cowpea (*Vigna unguiculata*). Each commodity was pre-equilibrated for three weeks prior to use (27±5°C, 60±5%rh). Subcultures using 200 – 300 adult beetles were set up from parental cultures. After their initial set-up, cultures were left in the CTH room for three weeks to enable the females to lay sufficient numbers of eggs. The adult beetles were removed from the culture and discarded to prevent confusion with the emerging F_1 population. *C. maculatus* were removed after ten days due to their shorter adult life-span of 7-10 days. The cultures were checked for the F_1 generation on the fourth week (*R. dominica* were checked at five weeks to reflect the average development time). After finding adult emergence of *P. truncatus*, *S. zeamais* and *R. dominica*, the adults were discarded, and the cultures were left for a further week. The cultures were then sieved after the week-long period, and newly emerged adult insects were placed on fresh commodity for one more week such that insects for experiments would be 7-14 days old. Due to their relatively short adult life, experiments using *C. maculatus* were conducted with insects that were 1-3 days old. After the initial sieving of *C. maculatus*, the culture was re-sieved 3 days later, and the insects that were collected were used for experimental testing.

Repellency bioassay

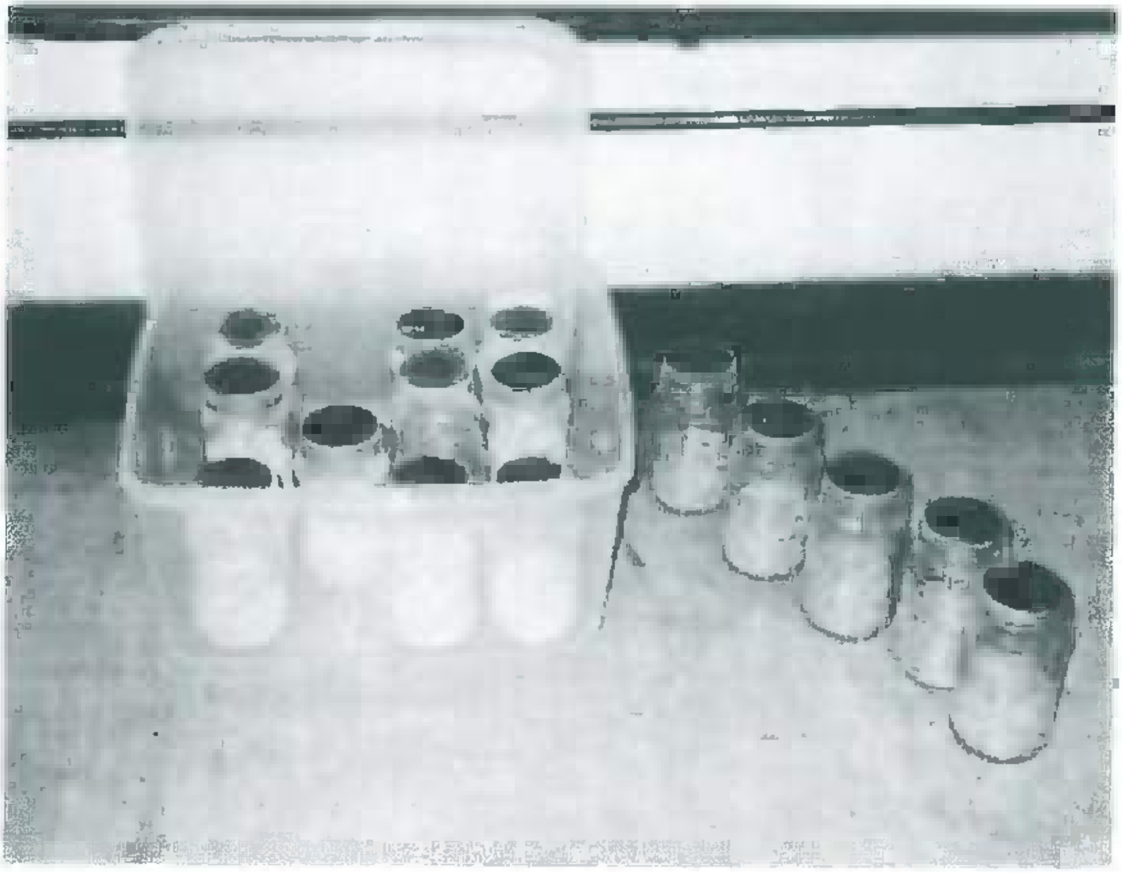
Repellency of a plant material to an insect was assessed using a choice preference arena. The arena consisted of a plastic box (323mm x 323mm x 158mm, Stewart Plastics Ltd.) with a cardboard sheet taped on the inside base. The cardboard assisted the insects to gain a footing on what would otherwise be a slippery surface. To prevent the escape of *C. maculatus* and *S. zeamais*, the inner inch at the top of the box was covered in fluon (polytetrafluoethylene) (Whitford Plastics Ltd.). Two 50g 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); control 'no-choice' replicates were two piles of untreated commodity. Four replicates were conducted for each treatment and insect tested. 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. The four replicate arenas were placed in a CTH room (27±5°C, 60±5%rh) so that the treated piles of commodity were concentric to each other, thus negating the potential influence of the room's lighting upon the direction of travel by the insects.

Toxicity bioassay

Pre-equilibrated commodity (100g, 27±5°C, 60±5%rh) was placed in 250ml glass jars, five replicates per treatment. Dried and pre-ground plant material was admixed (w/w) with the commodity at three different concentrations using a 1 litre hand-shaker jar (0.5%, 1.0% and 5.0%, plus untreated control). Forty known-age insects of one of the four test species were added to each jar with respect to their commodity type. The jars were then sealed with filter paper and molten wax and placed in a CTH room (27±5°C, 60±5%rh).

The toxicity bioassays were scored twice. An initial count was conducted to assess the mortality of the insects that had been originally placed in the experimental trials. For *C. maculatus* the initial count to record the number of alive and dead insects was performed 1 day after the bioassay was set up. Sieving for the first count was avoided with respect to the *C. maculatus* experiments because of the delicate nature of the eggs laid on the surface of the cowpeas. The initial counts for the remaining three insect species were obtained after 7 days. In order to count the number of alive and dead insects in these experiments, it was necessary to separate them from the host commodity by lightly sieving the trial jars. As a result, the plant material would require gentle re-mixing after the initial count was done. Scoring the final count of the number of alive and dead insects in the F_1 population was conducted at 28 days for *C. maculatus*; whereas, *P. truncatus*, *S. zeamais* and *R. dominica* were scored at 49 days.

Figure 1 **Example of the toxicity bioassays as maintained under controlled environment conditions (27±5°C, 60±5%rh)**



Results

Repellency bioassay

Results showed that *P. truncatus* was the most repelled by plant treated commodity followed by *R. dominica*, *C. maculatus*, and *S. zeamais* (Figures 2 to 5, 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, $P > 0.05$). *C. maculatus* was deterred by *Ocimum americanum* (kpasuik), *Khaya senegalensis* (kuga) and *Chamaecrista kirkii* (lodel) (Figure 2, Mann-Whitney U-tests between each treatment and the control, $Z > 2.4$, n = 4, $P < 0.05$). *S. zeamais* was only deterred by *Securidaca longipedunculata* (palaga) (Figure 3, Mann-Whitney U-tests between each treatment and the control, $Z > 2.4$, n = 4, $P < 0.05$). *R. dominica* was deterred by *Securidaca longipedunculata* (palaga), *Mitragyna inermis* (dekonja), *Cissus populnea* (cissus), *Capsicum annuum* (chilli pepper), *Chamaecrista kirkii* (lodel) and *Azadirachta indica* (neem) (Figure 4, Mann-Whitney U-tests between each treatment and the control, $Z > 2.4$, n = 4, $P < 0.05$). *P. truncatus* was deterred by all the plant materials tested (Figure 5, Mann-Whitney U-tests between each treatment and the control, $Z > 2.4$, n = 4, $P < 0.05$).

Figure 2 **Deterrency (%) of different botanicals admixed with commodity against *Callosobruchus maculatus***

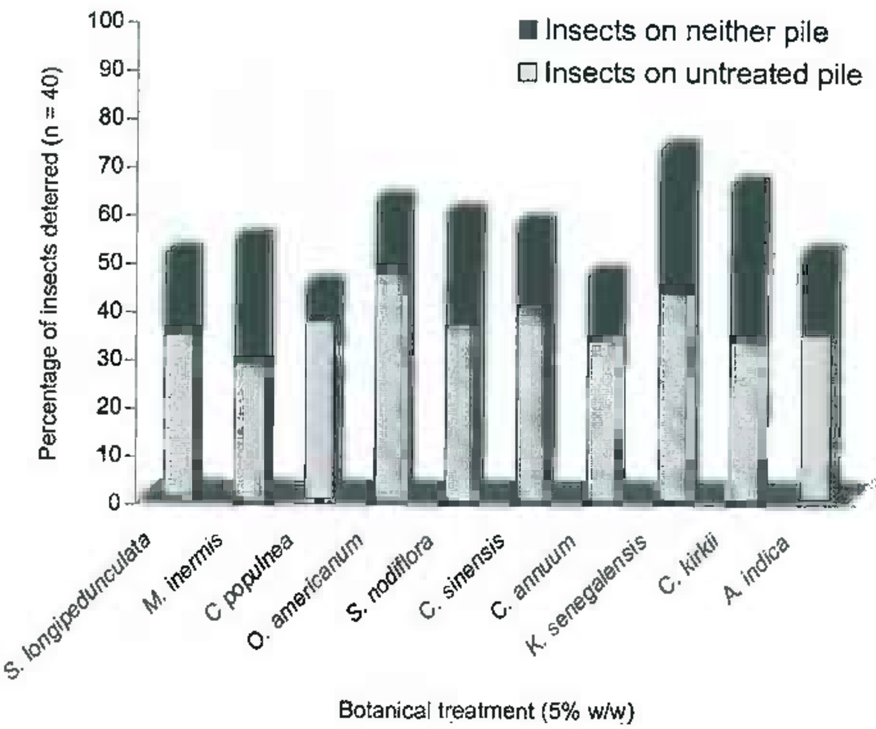


Figure 3 **Deterrency (%) of different botanicals admixed with commodity against *Sitophilus zeamais***

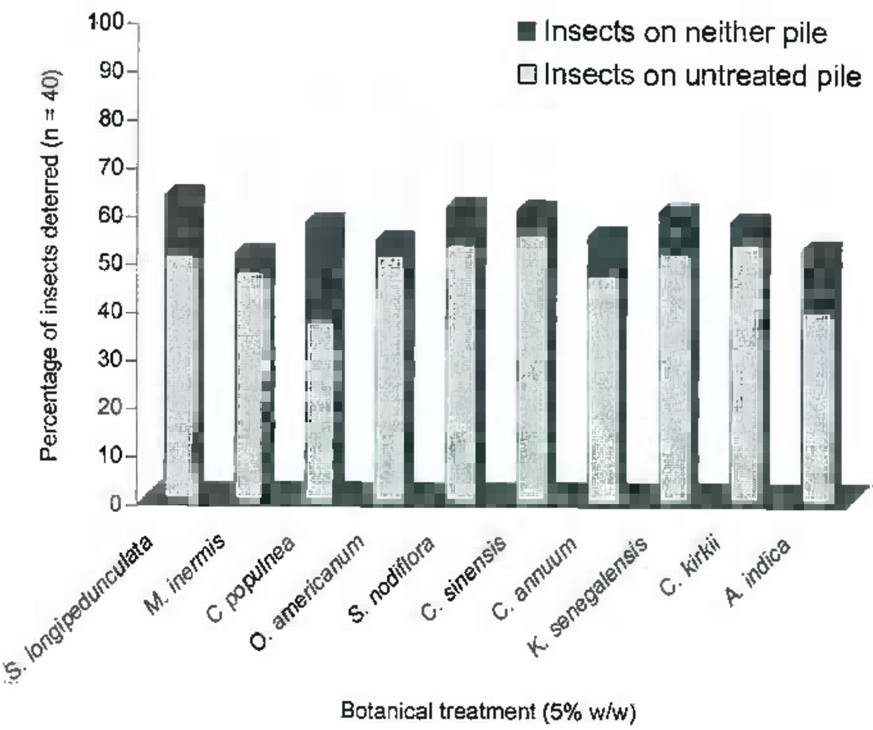


Figure 4 **Deterrency (%) of different botanicals admixed with commodity against *Rhyzopertha dominica***

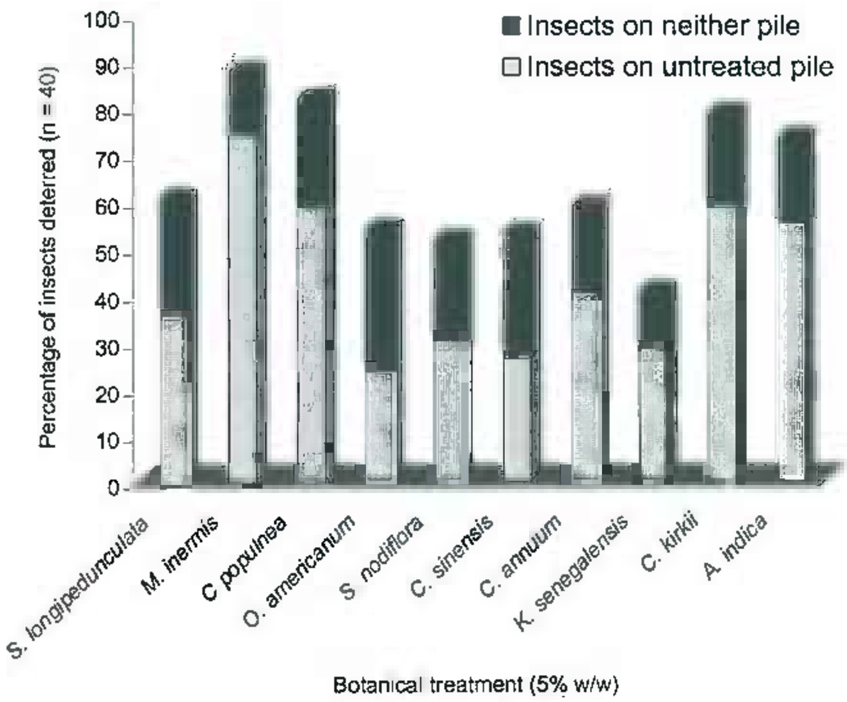
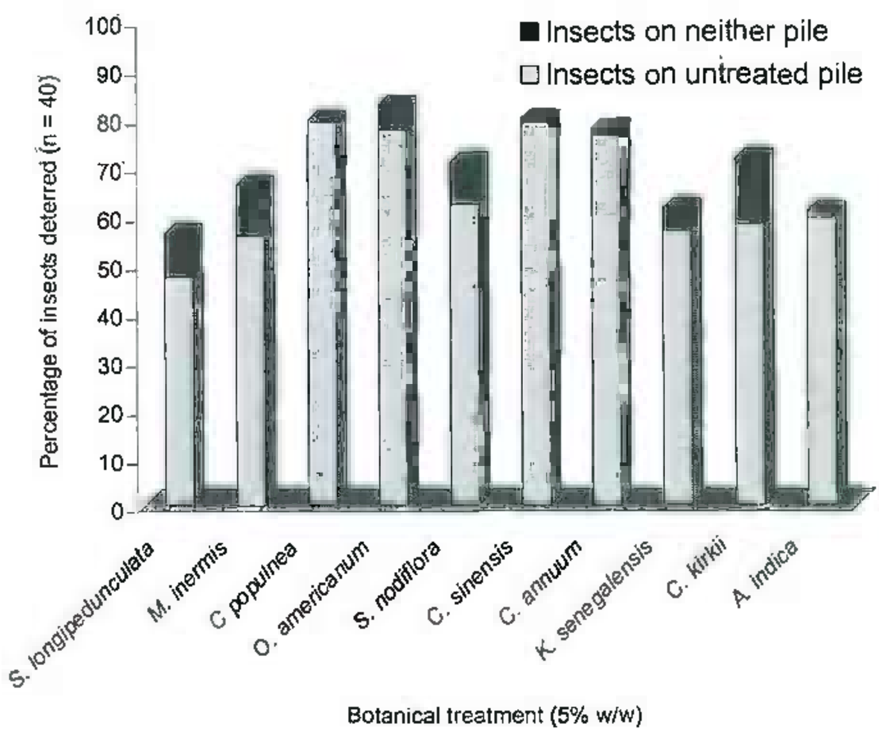


Figure 5 **Deterrency (%) of different botanicals admixed with commodity against *Prostephanus truncatus***



Toxicity bioassay

Results showed that *Securidaca longipedunculata* (palaga) was the most effective treatment for controlling the tested insects (Figures 6 and 7, Mann-Whitney U-tests alive and dead insects between each treatment and the control, $Z > 2.6$, $n = 5$, $P < 0.01$). The observed mortality and decreased F_1 emergence was dose-dependent, and percent mortality was as high as 80% in *R. dominica* trials at 5% w/w (Figure 7). 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 (Mann-Whitney U-tests alive insects between each treatment and the control, $Z > 2.6$, $n = 5$, $P < 0.01$). *Pleiocapa mutica* (kanbam) had a similar affect as *C. kirkii* against *R. dominica* and *S. zeamais* (Figure 8, Mann-Whitney U-tests alive insects between each treatment and the control, $Z > 2.6$, $n = 5$, $P < 0.01$). *Pterocarpus erinaceus* (nae) showed some ability to dose-dependently decrease the emergence numbers of *R. dominica* (Mann-Whitney U-tests alive insects between each treatment and the control, $Z > 2.6$, $n = 5$, $P < 0.01$). However, the 5% treatment of *P. erinaceus* was only able to decrease emergence from 400 insects in the control to slightly less than 300 insects, suggesting much higher concentrations of *P. erinaceus* would be required to have a conspicuous protective effect. *Grewia mollis* (youlaga) was shown to be effective by increasing mortality and decreasing emergence against all the insects. (Figure 9, Mann-Whitney U-tests alive and dead insects between each treatment and the control, $Z > 2.6$, $n = 5$, $P < 0.01$). *Ocimum americanum* (kpasuik) dose-dependently increased mortality in *P. truncatus*, *S. zeamais* and *C. maculatus* (Figures 10 and 11, Mann-Whitney U-tests alive and dead insects between each treatment and the control, $Z > 2.6$, $n = 5$, $P < 0.01$). *Mitragyna inermis* (dekonja) increased mortality in *R. dominica* and reduced emergence in *S. zeamais*, *P. truncatus* and *C. maculatus* (Mann-Whitney U-tests alive and dead insects between each treatment and the control, $Z > 2.6$, $n = 5$, $P < 0.01$). *Azadirachta indica* (neem) reduced emergence in *R. dominica*, *S. zeamais* and *P. truncatus* by as much as 50% (Figure 12, Mann-Whitney U-tests alive insects between each treatment and the control, $Z > 2.6$, $n = 5$, $P < 0.01$). *Capsicum annuum* (chilli pepper) reduced emergence in *C. maculatus*, *S. zeamais* and *R. dominica* (Figure 13, Mann-Whitney U-tests alive insects between each treatment and the control, $Z > 2.6$, $n = 5$, $P < 0.01$). Similar results were obtained with *Khaya senegalensis* for all the insects (kuga) (Figure 14, Mann-Whitney U-tests alive insects between each treatment and the control, $Z > 2.6$, $n = 5$, $P < 0.01$).

Figure 6 **Toxicity of *Securidaca longipedunculata* (palaga) against *Sitophilus zeamais* when powdered plant material was admixed with host commodity**

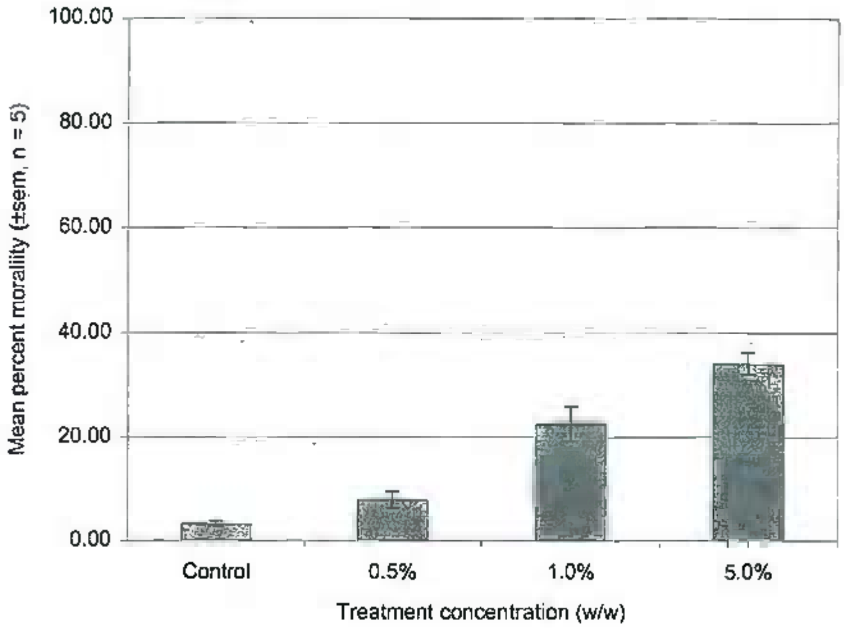


Figure 7 **Toxicity of *Securidaca longipedunculata* (palaga) against *Rhyzopertha dominica* when powdered plant material was admixed with host commodity**

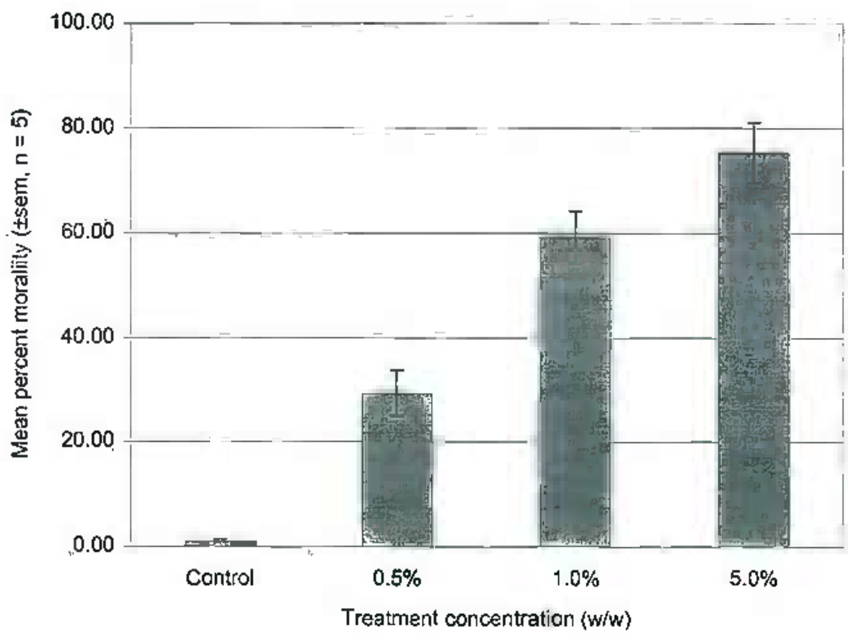


Figure 8 **Toxicity of *Pleiocapa mutica* (kanbam) against *Rhyzopertha dominica* when powdered plant material was admixed with host commodity**

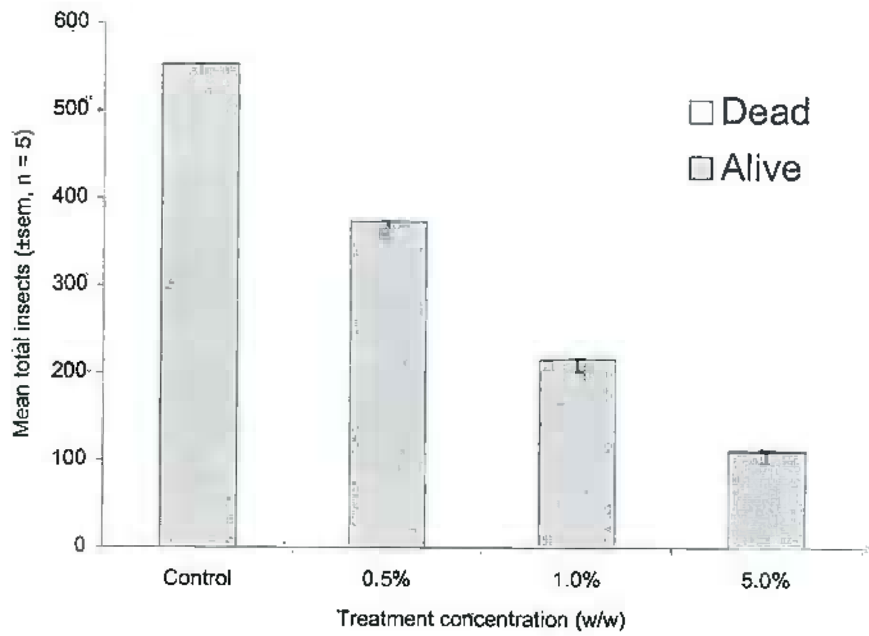


Figure 9 **Toxicity of *Grewia mollis* (youlaga) against *Sitophilus zeamais* when powdered plant material was admixed with host commodity**

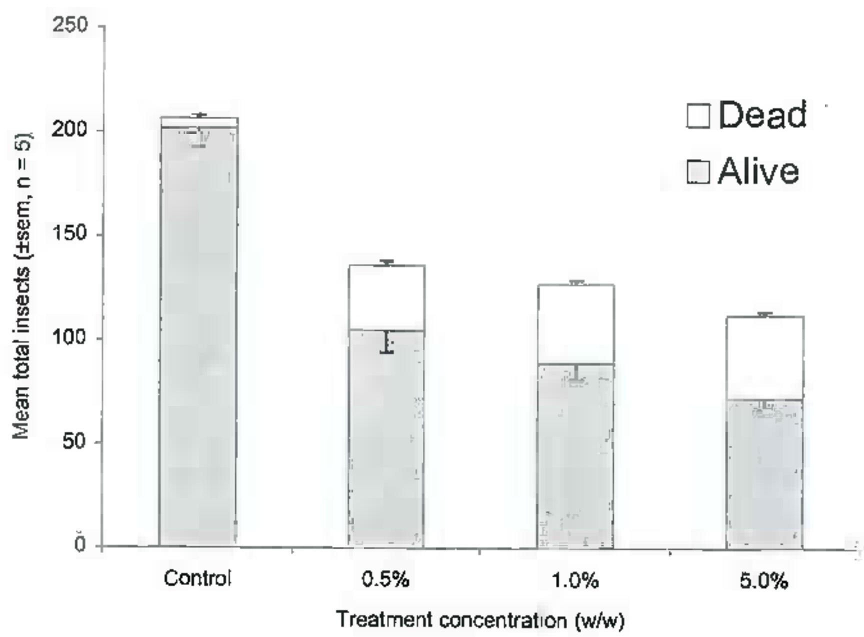


Figure 10 Toxicity of *Ocimum americanum* (kpasuik) against *Prostephanus truncatus* when powdered plant material was admixed with host commodity

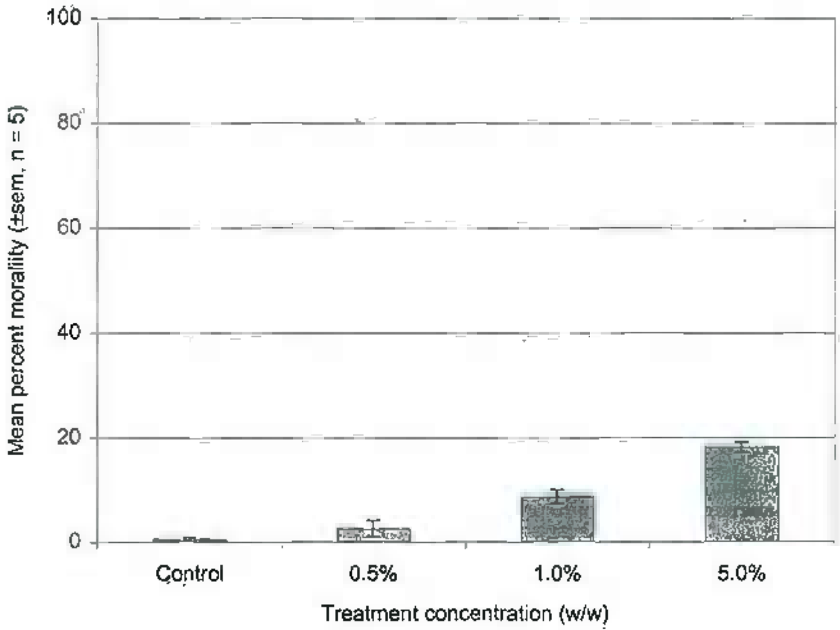


Figure 11 Toxicity of *Ocimum americanum* (kpasuik) against *Callosobruchus maculatus* when powdered plant material was admixed with host commodity

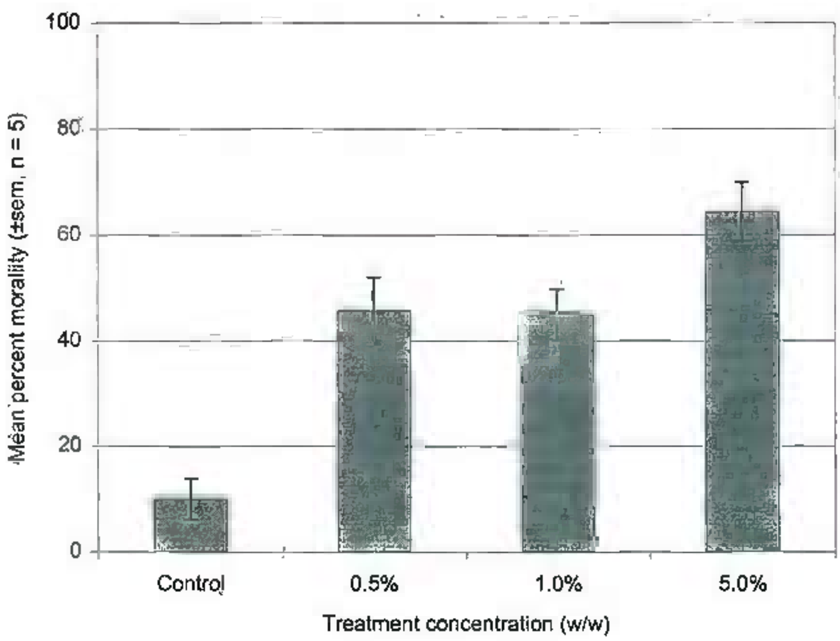


Figure 12 **Toxicity of *Azadirachta indica* (neem) against *Prostephanus truncatus* when powdered plant material was admixed with host commodity**

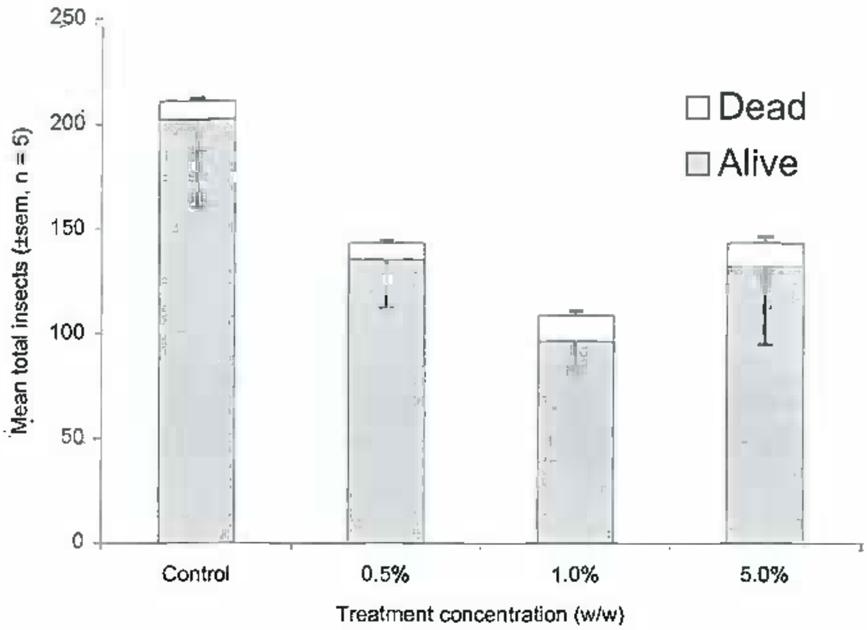


Figure 13 **Toxicity of *Capsicum annuum* (chilli pepper) against *Rhyzopertha dominica* when powdered plant material was admixed with host commodity**

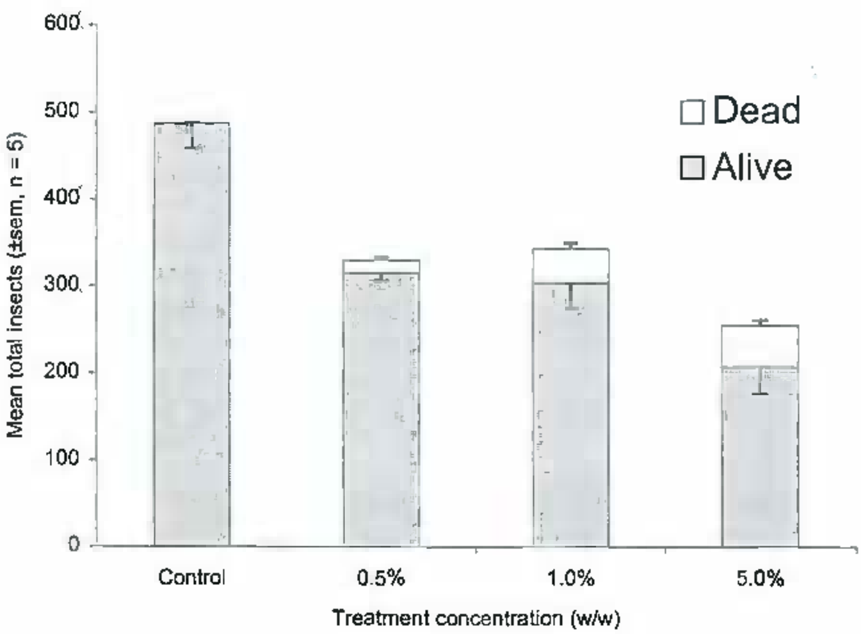
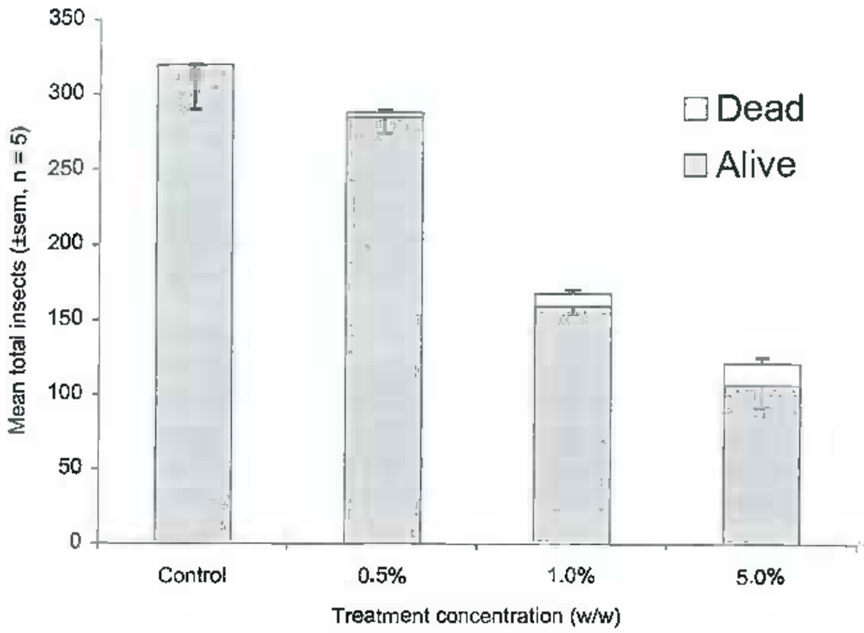


Figure 14 **Toxicity of *Khaya senegalensis* (kuga) against *Rhyzopertha dominica* when powdered plant material was admixed with host commodity**



Discussion

As expected, the efficacy of plant materials varied among the insect and plant species. The concentration application was important, and many of the plants showed classical dose-dependent effects that occur with conventional insecticides whereby increased dosage increased the toxic effect. The reasons for the observed variability among insect species' susceptibility to a plant material was not entirely clear. Generally, *C. maculatus* and *R. dominica* are considered to be more susceptible species when compared to *S. zeamais* and *P. truncatus*. These distinctions are based upon well-established physiological and behavioural differences associated with the insects. However, some of the plants appeared to be more effective against *S. zeamais* and *P. truncatus* than *C. maculatus* and *R. dominica*, such as *Grewia mollis*, *Azadirachta indica* and *Ocimum americanum*. These data would recommend that further research is conducted to confirm the results obtained. Some of the plants did not demonstrate increased percent mortality although they did decrease overall emergence of the F_1 generation. This would suggest that the plant's mode of action relates to another factor of the insect's life-cycle. For example, the plant may be toxic to the egg or larval stages or the plant may inhibit oviposition or interfere with mating, which would lead to an overall reduction in insect fitness.

The standardised application 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 this trial. Therefore, the potential toxicity associated with these plants may only become apparent with respect to differing application methods. Further bioassays as well as additional research on the phytochemistry of the plants will help confirm these observations.

Generally, the lowest concentration of plant material had no significant effect against the insects. Therefore, recommendations for on-farm use would suggest that concentrations of at least 1.0% are used, the higher the concentration the better protection provided. In most situations, this should be a realistic concentration to achieve, and some farmers that currently use plant materials would already be treating at concentrations of 1.0% or above.

The most effective plant material identified from these experiments, *Securidaca longipedunculata*, was not the most widely-used plant by farmers as assessed from the PRA survey conducted in Ghana. *S. longipedunculata* is a fairly common plant and can be found in many countries across Africa. Therefore, availability is unlikely to be the reason why farmers have not been using *S. longipedunculata*. Its limited use may be merely a function of limited knowledge system promotion, or it may be due to valid constraints to its use. Further on-farm participatory trials will be required to establish whether *S. longipedunculata* can be sustainably promoted. Other plant materials involved in the trial have also showed promising results, and they will require similar investigations to optimise their promotion at farm-level.

Similar toxicity experiments, although reduced in scope, were conducted with collaborators in Ghana at the Crops Research Institute. Data obtained at the CRI support the results presented above conducted at the NRI. The CRI research also showed that *S. longipedunculata* was the most effective protectant, and it was as effective as the synthetic Actellic that was used as a positive control group. The CRI results can be found in the report: OWUSU-AKYAW, M. (1999) Report on the use of indigenous plant products for the preservation of maize and cowpea seeds. Project R6501. Kumasi, Ghana: Crops Research Institute.

Field station trials

Introduction

The ecological implications of using plant materials as storage protectants are relatively complex when compared to simple laboratory trials testing for toxicity. Because field trials are more similar to the natural setting, field data could be considered more meaningful with respect to the practical implications of using plant materials as storage protectants. Although field trials which rely upon natural infestation mechanisms can often require lengthy experimental repetition over several seasons and large sample sizes, they are a crucial step towards scientific verification of the results. Although the work programme within the project prevented seasonal replication and large sample sizes, field trials were established to act as a preliminary comparison with laboratory trials as well as investigating the effects of application method upon overall efficacy.

Materials & methods

Field station trials were conducted with regional representatives of the Ministry of Food and Agriculture in Tamale and Bolgatanga. Trials in Tamale were conducted with three commodities, cowpea, maize and bambara and 15 different treatments (Table 2). Trials in Bolgatanga were conducted with cowpeas and sorghum and 9 treatments (Table 2). Outside these differences, experimental protocol was identical at both sites.

Experiments at each site were placed within four randomised block treatments, four replicates per treatment. The experimental blocks were placed together under a roof, but were otherwise open to environmental conditions and potential infestation. Each replicate consisted of 5kg of commodity placed within a clay pot that was left open at the top. Plant parts were collected following the methods used by farmers and then shade-dried. For dust treatments, plant materials were ground and admixed with commodity to the appropriate concentration (Table 2). For dip treatments, 1kg of plant material was added to 10 litres of boiling water and left for 30 minutes. Commodity was then dipped into the extract for approximately 20 seconds and then spread on a concrete surface to dry for 40 minutes before being placed in the clay pot. The kerosene and shea nut treatments were applied at 5ml/kg.

Table 2 **Description of treatments used during field station trials in Ghana. Plant dips are hot water extracts (app. 1 kg/10 l); dusts are admixed powdered material (w/w) (n = 4).**

Tamale, Northern Region	Bolgatanga, Upper East Region
Control – no treatment	Control – no treatment
Boiling water	Boiling water
Cold water	Famitatagba dip
Dekonja dip	Kimkim dip
Famitatagba dip	Kimkim dip and 1% kulenka dust
Kimkim dip	Kpasuik 0.4% dust
Kerosene	Kpasuik 3% dust
Kpasuik 1% dust	Kulenka 1% dust
Kulenka 1% dust	Lodel 1% dust
Lodel dip	Lodel 3% dust
Lodel 1% dust	
Palaga dip	
Palaga 1% dust	
Shea nut butter	
Shea nut residue	
Shea nut waste water dip	

Starting in March 1998, samples of 400g were taken from each replicate every six weeks. Final samples were collected in October 1998. Samples were sieved, and the number of alive and dead insects was recorded from each sample. Insect damage was assessed using 200 sub-sampled grains, and a 200g sub-sample was placed into a sealed plastic container to assess F_1 emergence after 30 days, upon which the number of alive and dead insects was counted.

Results

Commodity was not pest-free at the commencement of the trial. For example, cowpea samples taken at the beginning in March were, on average, 9% damaged and produced an average of 3.2 adults/100g. In all experiments, percent damage and F_1 emergence were positively correlated over time (Spearman Coefficient for each commodity > 0.77, n = 10, P < 0.01). Experiments conducted at Tamale and Bolgatanga showed that many of the treatments had no effect upon the level of damage incurred by storage pests in the four commodities tested (Figures 15 to 19). The efficacy of different treatments did not change with time over the six month period (Kruskal-Wallis for each treatment, $\chi^2 < 0.4$, df = 7, P > 0.05). Dips were more effective than dusts.

Experiments with cowpea at Tamale showed that boiling water, dekonja, famitatagba, kimkim, shea nut butter and lodel as a dip significantly reduced the percent insect damage (Figure 15, Mann-Whitney U-tests between each treatment and control, Z > 3.1, n = 28, P < 0.01). At Bolgatanga, the treatments found effective on cowpea were boiling water, famitatagba, kimkim and kimkim/kulenka (Figure 16, Mann-Whitney U-tests between each treatment and control, Z > 3.1, n = 28, P < 0.01). Boiling water was as effective as the hot water plant extracts in controlling insects on cowpea (Kruskal-Wallis among effective treatments, $\chi^2 = 0.5$, df = 9, P > 0.05). The only treatment found to significantly reduce infestation of maize was shea nut butter (Figure 17, Mann-Whitney U-test, Z > 3.1, n = 28, P < 0.01), and only the combined treatment of kimkim/kulenka reduced damage in sorghum (Figure 18, Mann-Whitney U-test, Z > 3.1, n = 28, P < 0.01). Dekonja, famitatagba and shea nut butter were effective in reducing percent damage of bambara (Figure 19, Mann-Whitney U-tests between each treatment and control, Z > 3.1, n = 28, P < 0.01).

Figure 15 **Tamale field trials testing the effect of different plant treatments upon the level of insect damage to cowpea over a six month period, March to October 1998.**

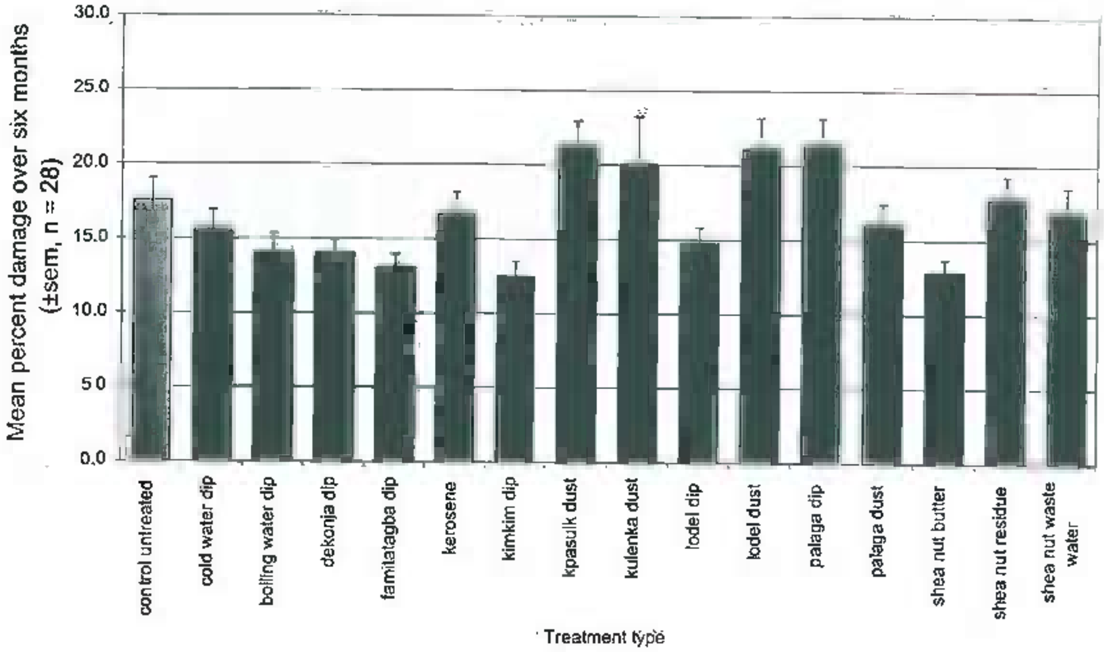


Figure 16 **Bolgatanga field trials testing the effect of different plant treatments upon the level of insect damage to cowpea over a six month period, March to October 1998.**

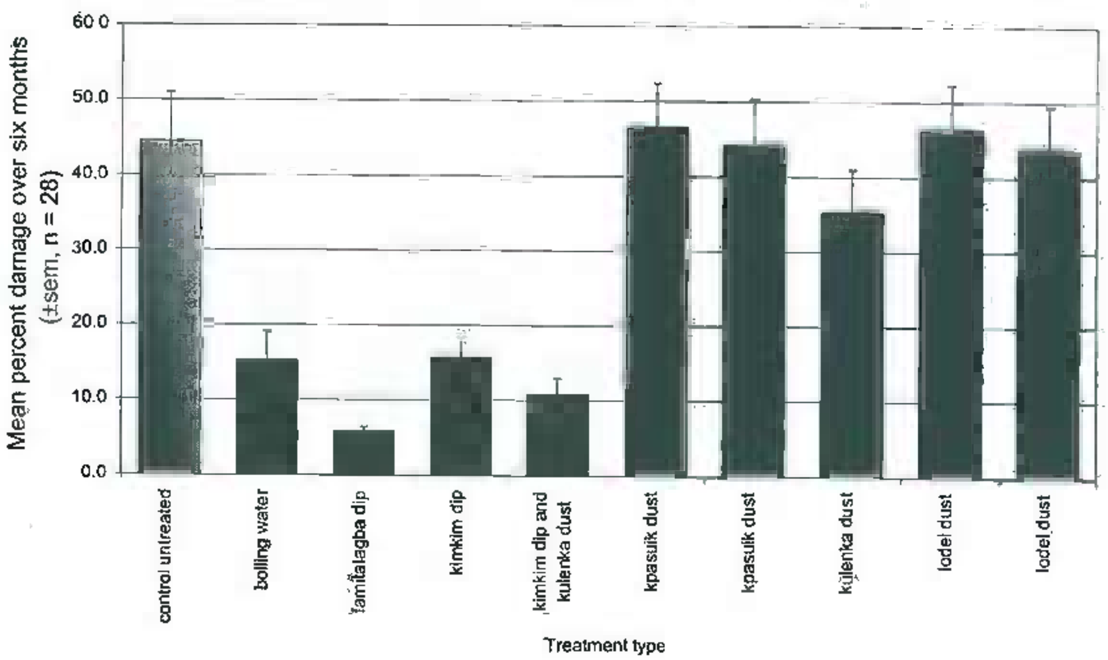


Figure 17 **Tamale field trials testing the effect of different plant treatments upon the level of insect damage to maize over a six month period, March to October 1998.**

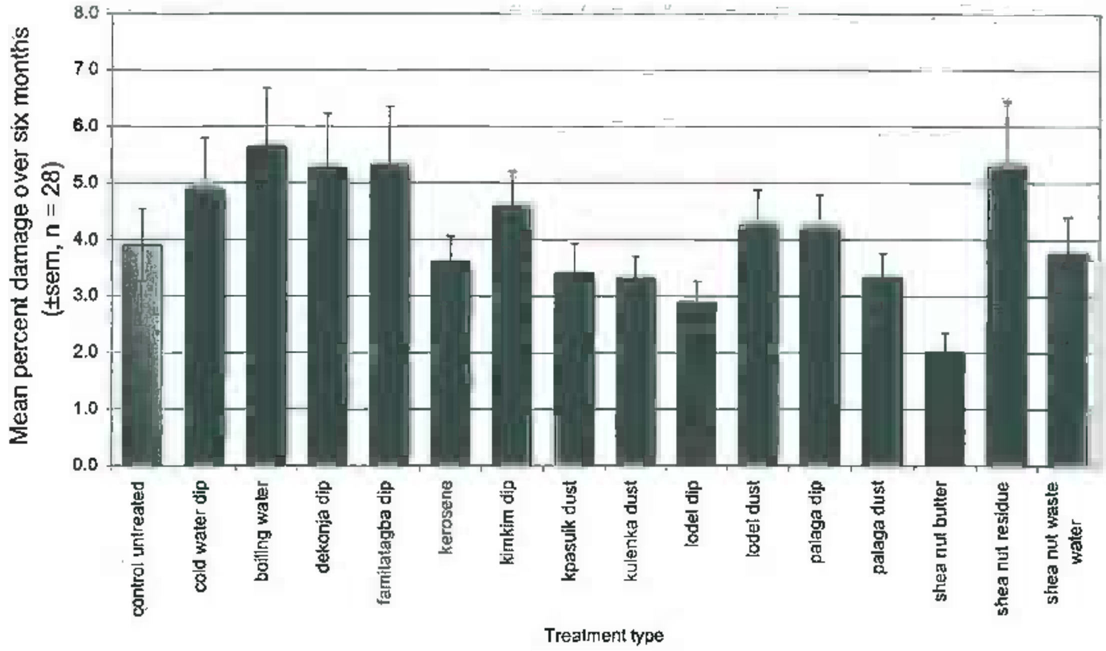


Figure 18 **Bolgatanga field trials testing the effect of different plant treatments upon the level of insect damage to sorghum over a six month period, March to October 1998.**

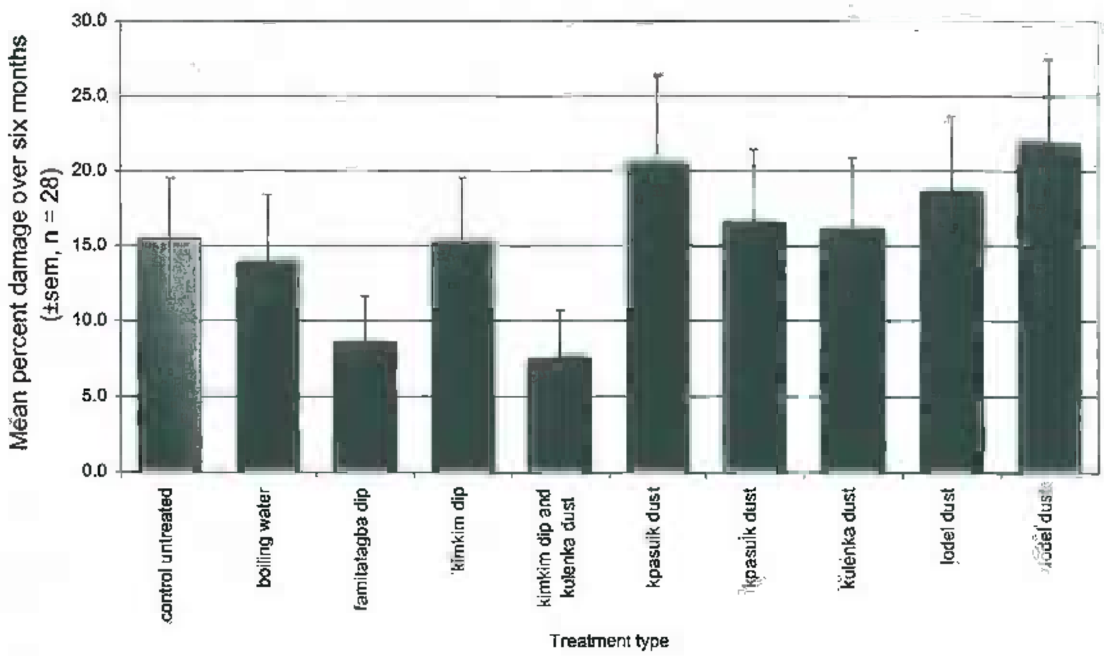
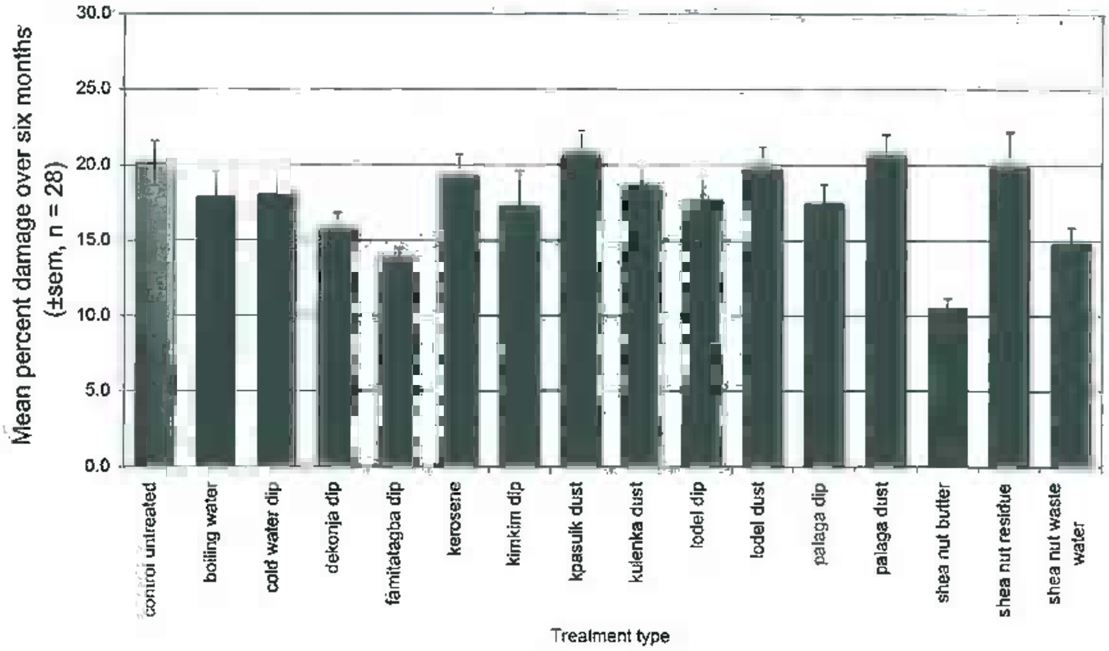


Figure 19 **Tamale field trials testing the effect of different plant treatments upon the level of insect damage to bambara over a six month period, March to October 1998.**



Discussion

Results from the station trials conflict with results obtained from laboratory toxicity trials conducted in Ghana and the UK. For example, the plant material *S. longipedunculata* (palaga) was extremely effective in controlling storage insects in laboratory trials, but appeared to provide no protection during field trials. There are several speculative explanations for these discrepancies. Firstly, plant materials are known to vary in the quantity and quality of secondary metabolites responsible for bioactivity. Therefore, different batches of plant material could result in different levels of efficacy. Similarly, the dust concentrations used for the field trials were potentially lower than required to provide protection. A more likely explanation is related to the experimental methodology of the field trials. Because control replicates were dispersed throughout the treatments without any barriers to cross contamination, pest populations could have repeatedly infested treatments. The equivalent situation in a farmer store would be when a farmer only treats part of the stored commodity, leaving adjacent stock untreated. This would be bound to increase the infestation level found in the treated commodity. Due to workload obligations, only four replicates could be managed by MoFA staff during the station trials. This protocol led to high variability within and among treatments and reduced the statistical comparability of the results. Future experiments will need to increase the number of replicates as well as address variability factors across seasons.

With a few exceptions, plant materials that are more intensively used by farmers for storage appear to be the better protectants as found from laboratory trials. Therefore, efficacy results from laboratory toxicity trials roughly follow farmer practice. This would suggest that the field station trials should be repeated to verify the results obtained. Application method, e.g. dips and dusts, showed that dipping the commodity (especially cowpea) in hot water was effective in reducing infestation. This could be due to the hot water killing off any initial infestation, and not necessarily due to the effects of the plant material extracted into the water. Clearly, more research is required to establish what factors influence the efficacy of plant materials, i.e. application method or concentration, phytochemistry variability, plant collection time, etc. These issues are recommended for continued research in order to more widely adapt and promote the usage of plant materials during on-farm storage.

Rodent feeding trials

Introduction

It could be assumed that farmers have been using the plants identified from the PRA survey as storage protectants for generations with no ill effects. This may be because there has been some previous selection occurring with regard to safety, i.e. farmers eliminating highly toxic plants from use as storage protectants. Or it may also be that commodity is washed and cooked before consumption leaving little plant material residue behind to be ingested.

Earlier project activities involving database searches on the plant species showed that there was little information available on the plants' phytochemistry or mode of action. This lack of existing research would make it difficult to institutionally promote insecticidal plants as they could affect the health of farmers who use them. In collaboration with the Medical Research Council, UK, some of the identified plants were screened using rodent feeding assays to determine whether the plants could be harmful to humans or livestock.

Materials & methods

Six plant materials were incorporated into rat diet at 1% and 5% (w/w) and fed to rodents over a six week period. (Table 3). 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 two weeks prior to trial commencement. The parameters measured during the trial were dietary intake, growth rate and rodent behaviour. The behavioural parameters were assessed at trial mid-point and at trial completion and included: appearance, grooming, incontinence, alertness, muscle tone, handling response, startle response, righting reflex, gait, gross tremor, twitches, aggression, fearfulness and repetitiveness. At the end of the trial, kidneys and livers were visually and histologically examined.

Table 3 **Plant species used for the rodent feeding assay**

Local name	Latin name
Lodel	*Chamaecrista kirkii*
Cissus	*Cissus populnea*
Dekonja	*Mitragyna inermis*
Kpasiuk	*Ocimum americanum* var *ocimum*
Palaga	*Securidaca longipedunculata*
Kimkim	*Synedrella nodiflora*

Results

Dietary intake and weight gain were normal in all treatments except for palaga fed at 5% and lodel fed at 5% (Figure 20). None of the treatments, including palaga and lodel, affected weight gain when fed at 1% (Figure 21). The reduced weight gain associated with palaga 5% and lodel 5% was not low enough to necessitate a change in experimental protocol to include pair feeding.

Behavioural assessments of rats showed no abnormalities. Rats were considered to be happy and normal at trial mid-point and at the end of the six weeks.

On post-mortem examination, livers and kidneys appeared normal with exception of palaga 5% where kidneys were pale and small. The weights of livers and kidneys were normal with the exception of palaga 5% and lodel 5%. Kidney pathology showed no significant findings with the exception of palaga 5% where reduced accumulation of α-2μ-globulin was observed. Liver pathology showed increased mitotic division in palaga 5% and lodel 5%. Hyperplasia in the liver was due to increased cell death, and all 5% treatments and some 1% treatments showed increased mitosis.

Further results of the trial can be found in the report: NEAL, D.E. (1998) Final report on rodent feeding trials testing plant materials for potential vertebrate toxicity. Project R6501. Leicester, UK: Toxicology Unit, Medical Research Council, University of Leicester.

Figure 20 **Effect of six plant materials upon rodent growth rate when incorporated into their diet at 5% (w/w, n = 6). Bars marked with * are significantly different from the control, Mann-Whitney U-test, P < 0.05.**

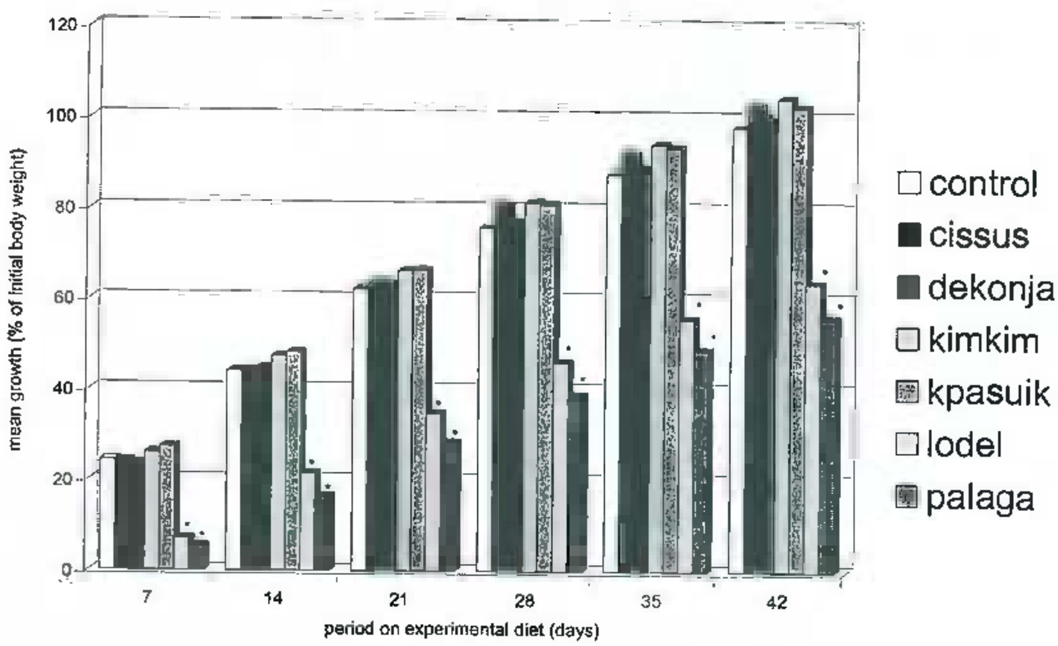
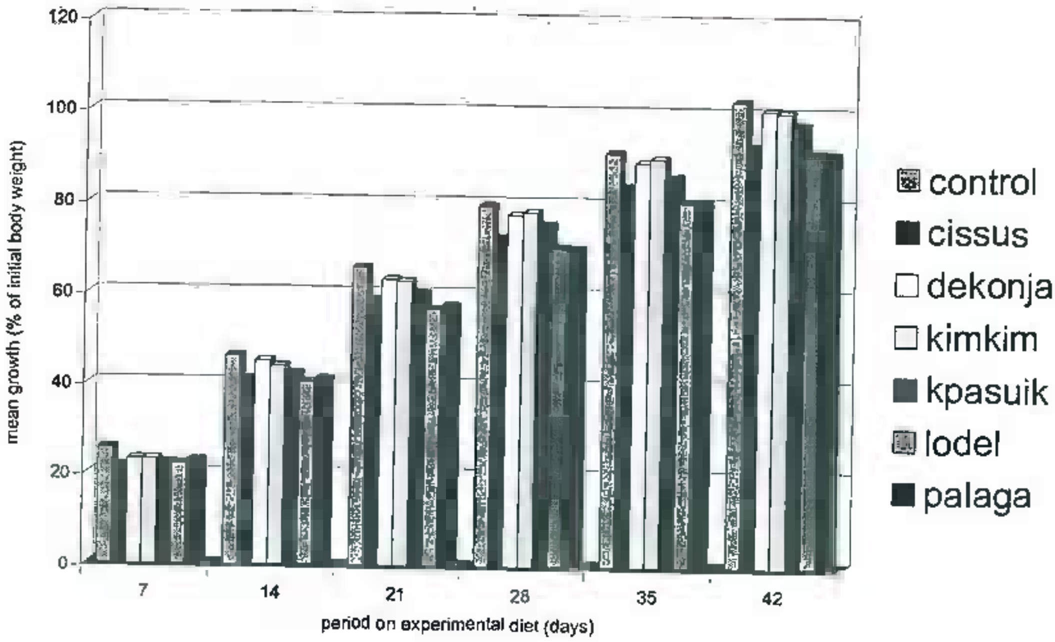


Figure 21 **Effect of six plant materials upon rodent growth rate when incorporated into their diet at 1% (w/w, n = 6). No treatments were significantly different from the control, Mann-Whitney U-test, P < 0.05.**



Discussion

Results showed that the plants were only mildly toxic to rodents over the six week feeding period. Even in the most adversely affected groups of 5% palaga and 5% lodel, the rodents continued to consume enough of the diet to fall within normal growth parameters. Behavioural assessments showed that there were no obvious neurological effects and that rodents appeared healthy. Kidney pathology showed there was an effect by palaga 5% through the accumulation of α-2μ-globulin. Although this biochemical pathway is specific to rodent species, there could be implications with respect to human kidneys through another mechanism. Many of the treatments caused increased liver regeneration. These results do not directly imply the plants were toxic, but increased mitotic activity could increase the opportunity for cancer development. Prolonged feeding trials would be required to demonstrate any potential chronic effects of the plants.

In conclusion, the rodent feeding assays would suggest that the plants are safe to use as storage protectants. Only some of the 5% concentrations showed any slight negative effect during this trial, but prolonged trials could possibly show effects at lower concentrations. However, the actual amounts of plant material that would be potentially ingested by humans or livestock would be much less than the test concentrations of 1% and 5%. This is because the farmer would remove the plant material before consumption through such processes as washing. However, it is not known to what extent plant residues may be left behind after normal pre-consumption processes. Future research should attempt to measure potential residues left on plant-treated grain after storage and determine whether there are any potential effects from these residues.

On-farm trials

Introduction

The plant materials that have been involved in the laboratory and field trials in this project were initially identified by farmers who already use them as storage protectants. The results from the above trials showed that some of the plants commonly used by farmers were not that good in controlling pests under controlled conditions. Therefore, one purpose of the on-farm trials was to study the plant materials under the conditions of normal use by farmers. This could help confirm any discrepancies among the different experimental methodologies that may not account for all the control properties. Farmer participatory trials would also give opportunity to explain and disseminate information as well as gain farmer feedback on which plants are preferred, identifying the advantages and constraints associated with different plant species.

Materials & methods

Prior to commencement of the trials, MoFA collaborators and district extension agents identified suitable villages to take part in the trial. The criteria were that sufficient numbers of farmers in the villages could be found who normally store maize and cowpea for at least six months and that the farmers would be happy for the extension agent to periodically remove small samples from their store for analysis over the duration of the trial. Extension agents provided a list of farmers who met these criteria from which names of farmers were randomly chosen to be included in the trial.

Trials were set up at the beginning of the storage season in December 1998. Maize trials took place in the Tolon District, Northern Region, as this is a major maize growing area. Seventy farmers were involved in testing six different plant treatments (Table 4). Twenty kilograms of the farmer's own maize was placed in a polythene sack and one of the six plant treatments was randomly assigned (Figure 22). At least five of the farmers from each plant treatment group stored an additional 5kg of maize in a separate polythene sack to act as an untreated control. Control sack assignment was based upon those farmers who had sufficient commodity to make a control sack. Plant materials used by farmers as dusts, i.e. kulenka, kpasuik, palaga and lodel, were admixed at a concentration of 1% w/w by adding 200g of plant material and then mixing the maize and plant dust in the sack. Plant materials as dips were carried out according to the methods used by farmers by boiling the plant in water, and then dipping the commodity into the extract. The commodity was then spread out on a concrete surface and dried before being placed in the sack (see cover illustrations showing the process). Trials involving cowpea followed a similar protocol with the following modifications. Trials were conducted in the Zebila District, Upper East Region, where sufficient farmers grow adequate quantities of cowpea. Sixty farmers were involved in testing four different plant treatments including a separate group of farmers storing untreated commodity as a control (Table 4).

Farmers were asked to store the polythene sack of commodity within their normal storage facility with the rest of their stock. Samples of 200g were collected every six weeks over a six month period (December 1998 to May 1999) from treatment and control sacks. Percent damage, the number of adult insects and the F_1 emergence number were recorded for each sample.

Table 4 **Treatment types used during the on-farm trials**

Northern Region maize trials	Upper East Region cowpea trials
Kpasuik dust (1% w/w) + control	Kpasuik dust (1% w/w)
Kulenka dust (1% w/w) + control	Kulenka dust (1% w/w)
Palaga dust (1% w/w) + control	Lodel dust (1% w/w)
Lodel dust (1% w/w) + control	Kimkim dip
Dekonja dip + control	Control – no treatment
Famitatagba dip + control	

Figure 22 **Plant material being added to polythene sacks of commodity before distribution to farmers involved in the trial**

Results

Because the on-farm trials are due to complete at the end of the storage season in May 1999, the final results cannot be included within this technical report. As expected, initial results are showing variable efficacy among different treatments. Cowpea damage at the commencement of the trial was already quite high (app. 5-15%), and this will affect the ability of the plant materials to protect the commodity and reduce infestation. Maize damage was very low at trial commencement, although moisture content appeared to be highly variable among farmers which could lead to future infestation. The final assessments for these on-farm trials have been included within a proposed project that will continue research on optimising plant material usage as storage protectants. Results of the on-farm trials and the associated farmer feedback will allow recommendations to be formulated on which plant materials could be used at farm level and/or require further research.

Discussion

On-farm experimental trials in the Northern and Upper East Regions of Ghana have given some farmers first-hand experience in using plant materials to treat maize and cowpea, many of whom had never previously used plants materials as storage protectants. The farmers who took part were very curious and enthusiastic about the work and saw that plant materials could offer practical solutions to their storage problems. Although many of the farmers may not have been using plants specifically as storage protectants, all farmers were familiar with various other medicinal or insecticidal properties of plants. Some of the plants involved in the trial were recognised by farmers as having medicinal properties as well. The farmers, therefore, felt comfortable with using plants as storage protectants and wanted to know more about the plants being used in the trial. They also raised many relevant questions, which we were not always able to answer. For example, farmers wanted to know if the plant treatments would affect seed germination and viability, for which no current research is available. Some of the farmers asked if they could try planting some of the treated grain during the next season to see how well it germinated. Farmer feedback regarding any potential tainting of grain on consumption will also help determine which plant materials are more likely to be widely promoted. In conclusion, the on-farm trials have contributed towards increased information dissemination to the end users as well as providing essential scientific information on the efficacy and acceptability of plant materials as storage protectants under realistic conditions.

Outputs

Plants with protective properties against insect infestation identified

A participatory rural assessment showed that all farmers surveyed favourably rated the use of plant materials in comparison to commercial synthetics. Seventeen different plant species were identified as currently used by farmers as storage protectants, eight of which were commonly used throughout the northern areas of Ghana. The farmer assessments of marker criteria (cost, effectiveness, availability, toxicity, ease of use, acceptability and versatility) for each plant species showed that some plant materials were preferred over others, either because of their availability or their ease of use.

The project showed that 74% of villages visited during the survey showed some usage of plant materials as storage protectants. However, out of the three regions surveyed (Northern, Upper East and Upper West), the usage of plant materials was geographically biased towards the Upper East where the overall number of farmers using plant materials was highest. The current usage of plant materials appears to be ethnically and culturally biased, constraining the availability of indigenous knowledge systems.

Recommendations for on-farm use of plant materials in grain protection

Vertebrate toxicity was negligible when plant materials were incorporated into rodent diets over a six-week period. However, two plant materials, *Securidaca longipedunculata* and *Chamaecrista kirkii*, gave some indication they could be harmful in the unlikely event they were ingested at high concentrations over prolonged periods of time.

Laboratory and field trials showed that the most effective plant material in controlling storage insects was *Securidaca longipedunculata*. Some of the other plant materials tested showed toxicity (*Chamaecrista kirkii*, *Azadirachta indica*, *Khaya senegalensis*, *Capsicum annuum Ocimum americanum*, *Grewia mollis*, *Pterocarpus erinaceus*, *Mitragyna inermis*, *Pleiocapa mutica*) or repellency (*Cissus populnea*, *Citrus sinensis*, *Securidaca longipedunculata*, *Chamaecrista kirkii, Khaya senegalensis, Mitragyna inermis, Azadirachta indica, Capsicum annuum*) against some or all of the insect species tested. Control levels were influenced by the method of application and concentration used. Dipping commodity into a hot water extract appeared to be more effective than admixing powdered plant material. The final assessments of ongoing farmer trials will allow recommendations to be formulated on which plant materials could be used at farm level and/or require further research.

Contribution of Outputs

The outputs have provided indicators as to what further initiatives are required in order to increase the usage of locally available plant materials and have identified potential constraints to their uptake. Over-collection pressure could become a constraining factor if the identified plants were widely used. However, many of the plants could be easily propagated if such pressures became evident, whereas others are highly abundant weed species that are unlikely to reach over-utilisation. It is important that issues involving the potential detrimental effects on vertebrates are addressed in future research. It remains unknown if plant residues are left behind on treated commodity before consumption and whether these potential residues would be found in sufficient quantities to cause any deleterious effects.

It is clear that further research including more farmer participatory trials and phytochemical analysis will be required to widely promote plant materials as storage protectants. In order to increase the usage of plant materials, farmers must be given the assurance of what level of protection is provided when a certain plant is used in a certain way. The major constraint to this provision is knowledge about the plant materials' chemistry and mode of action. Plants are known to vary their phytochemical content in response to environmental conditions. This will affect the quality and quantity of control that a farmer could expect. Providing farmers with information on what plants are best, when and how the plants are best collected and prepared will lead to increased reliability of control, giving resource-poor farmers a strong weapon to reduce deterioration of their stored commodity in an environmentally sustainable way.

Project Reports

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OWUSU-AKYAW, M. (1999) Report on the use of indigenous plant products for the preservation of maize and cowpea seeds. Project R6501. Kumasi, Ghana: Crops Research Institute.

Other Dissemination:
On-farm experimental trials in the Northern and Upper East Regions of Ghana have given some farmers first-hand experience in using plant materials to treat maize and cowpea, many of whom had never previously used plants materials as storage protectants. This direct dissemination of information was positively received by the end-users.

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[1] The views expressed are not necessarily those of DFID.