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Comparative study of field and laboratory evaluations of the ethnobotanical *Cassia sophera* L. (Leguminosae) for bioactivity against the storage pests *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) and *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae)

Cristina Kestenholz[a], Philip C. Stevenson[a,b,*], Steven R. Belmain[a]

[a]*Natural Resources Institute, University of Greenwich, Chatham Maritime, Kent ME4 4TB, UK*
[b]*Jodrell Laboratory, Royal Botanic Gardens, Kew, Surrey TW9 3DS, UK*

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Abstract

The powdered leaves of *Cassia sophera* along with hot- and cold-water leaf extracts of this plant were tested in laboratory experiments in the UK and in field trials in Tamale, Northern Ghana, using traditional storage containers, to determine their inhibitory and toxic effects against *Sitophilus oryzae* and *Callosobruchus maculatus* infestation of stored rice and cowpea, respectively. Laboratory and field experiments with cowpea showed that the use of *C. sophera* hot-water extracts was more effective at reducing *C. maculatus* infestation and adult emergence on cowpea than the traditional leaf-powder application (1% and 5% w/w) or the use of a cold-water extract of *C. sophera*. Hot-water extracts of *C. sophera* might be a more effective technique of applying the plant material on to stored cowpea than using powdered *C. sophera* leaves, the currently used application by small-scale farmers. In contrast, experiments with *S. oryzae* on rice showed that *C. sophera* leaf powder (5% w/w) effectively reduced adult emergence in the laboratory, but this could not be confirmed under field conditions. The hot and dry climatic conditions in the field might impart a natural protection against rice infestation by *S. oryzae*, making the use of protectants and pesticides less necessary for farmers. This was supported by the negligible rice grain damage after 6 months of field storage and by the failure of the *S. oryzae* population to establish itself under field conditions. The implications of using botanicals in pest control are discussed.
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1. Introduction

The use of species of the genus *Cassia*, such as *C. nigricans* Vahl, *C. occidentalis* L. or *C. siamea* Lam. as protectants of stored legumes has been reported by Babu et al.(1999), Dwivedi and Maheshwari (1996), and Lambert et al. (1985). *Cassia sophera* L., which is distributed throughout the tropics, is traditionally used by subsistence farmers in northern Ghana to protect stored cowpea (*Vigna unguiculata* (L.) Walp.), bambara groundnuts (*Vigna subterranea* L.), millet (*Pennisetum glaucum* L.), sorghum (*Sorghum bicolor* L. Moench) and maize (*Zea mais* L.) against insect infestation. It is traditionally used as powder obtained by pounding the dried leaves and mixing with the stored commodity (Belmain et al., 1999). The species is abundant and widespread, grows along roadsides and on waste ground and is reported to be a common weed in uncultivated lands (Belmain and Stevenson, 2001). In laboratory experiments, dry leaf powder of *C. sophera* increased adult mortality of *Callosobruchus maculatus* (F.) and *Rhyzopertha dominica* (F.) when admixed at 5% w/w to cowpea or wheat (*Triticum aestivum* L.), respectively (Belmain et al., 2001). In the same study, 1% and 5% concentrations of the leaf powder also significantly reduced F1 adult emergence of *C. maculatus*, *R. dominica* and

*Corresponding author. Tel.: +44 0 1634 883212; fax: +44 0 1634 883379.
E-mail address: P.C.Stevenson@gre.ac.uk (P.C. Stevenson).

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Sitophilus zeamais Motsch. on cowpea, wheat and maize, respectively. In addition, the 5% w/w leaf powder of *C. sophera* was reportedly repellent towards *R. dominica* and *Prostephanus truncatus* (Horn) when insects were given the choice between treated and untreated commodities (Belmain et al., 1999).

In order to improve recommendations to farmers in northern Ghana about the use of *C. sophera* as a storage protectant, laboratory and field experiments with traditional storage containers were conducted to validate the use and compare the efficacy of different application methods of the plant material. Little research evaluating botanicals has attempted to assess bioactivity under field conditions and has instead focussed predominantly on laboratory work that does not always give useful results. Under field conditions a wide range of variables, such as temperature, humidity, grain quality, storage environment, previous or existing infestation, commodity variety or different insect species and biotypes might influence the efficacy of plant-based pesticides. Before giving any recommendations to farmers about the use of plant-based pesticides and repellents, it is therefore essential to verify laboratory results under field conditions (Songa and Rono, 1998). The targeted species in this study were *C. maculatus* on cowpea and *Sitophilus oryzae* on rice. Rice is gaining increasing importance as a staple food in West Africa (Djayeola, 2000). This trend can be observed in Ghana but, in comparison to cowpea, little attention has been given to traditional storage techniques of rice. Therefore, this study introduces an innovative approach to the use of traditional plant-based pesticides for rice in Ghana.

2. Materials and methods

2.1. Plant material

Cassia sophera was collected in Ghana, Northern District, between September and November 1998. The leaves were shade-dried and ground to a powder in a two-stroke engine hammer mill commonly used by local people in Tamale, Ghana, for grinding commodities. Cowpea and brown rice used in the laboratory were purchased from wholesalers in the UK (Gillet A. Cook, Faversham, UK, and Canterbury Wholefoods, Canterbury, UK), frozen for 7 days and equilibrated to 14.5% moisture content in a room with controlled temperature and humidity set at $28\pm1\,^{\circ}C$, $58\pm10\%$ r.h. (12 h light cycle) and $25.5\pm1\,^{\circ}C$, $45\pm10\%$ r.h. (12 h dark cycle). Brown rice and cowpea used under field conditions were purchased at Tamale market, Northern Ghana, and fumigated with phosphine for 5 days prior to their use in experiments to ensure insect-free commodity.

2.2. Insect material

Callosobruchus maculatus and *S. oryzae* cultures were reared on cowpea and brown rice, respectively, under controlled temperature and humidity at $28\pm1\,^{\circ}C$, $58\pm10\%$ r.h. (12 h light cycle) and $25.5\pm1\,^{\circ}C$, $45\pm10\%$ r.h. (12 h dark cycle). The same strains of *C. maculatus* and *S. oryzae*, in culture since 1996, were used for all laboratory studies and to infest cowpea and rice used in the field experiment.

2.3. Commodity treatment

Rice and cowpea were mixed either with ground *C. sophera* leaves at concentrations of 1% and 5% w/w or treated with hot- and cold-water extracts of *C. sophera*. Organic solvent extracts were not investigated, as these solvents would not be readily available to Ghanaian farmers. The hot-water extract used in the laboratory was obtained by adding 100 g of *C. sophera* leaf powder to 800 ml of boiling distilled water (12.5% w/v), boiled for 15 min and subsequently filtered through a muslin cloth. The cold-water extract was obtained using the same procedure, except that the plant material was extracted for 2 h in cold water before filtering. A longer extraction time was used for cold-water extracts to compensate for the lower rate of solution of compounds in water at lower temperature. After filtering, grain used under laboratory conditions was immediately dipped in hot- or cold-water extracts for 10 s and dried on top of filter paper for 1 h in a fume cupboard.

The amount of plant material used to prepare hot- and cold-water extracts in the field experiment was chosen according to the protocol of local farmers, using a reduced concentration of 8% w/v, compared to the 12.5% used in the laboratory. The hot-water extract used for the field experiment was prepared by boiling 2 kg of *C. sophera* whole dry leaves and stems in 25 l of water (8% w/v) for 10 min, after which the plant material was removed. The cold-water extract was obtained using a similar procedure, except that the plant material was soaked for 12 h overnight in 25 l of cold water before using the extract the next day. Grains used in the field experiment were dipped in hot- or cold-water extracts twice consecutively for 5 s and subsequently spread on top of plastic sheets to dry for 90 min before using them in the experiment. Separate extracts were used to coat rice and cowpea. Controls consisted of untreated grains and grains treated with hot or cold water alone.

2.4. Laboratory experiment

Two experiments, one with rice and one with cowpea, were set up by introducing 20 unsexed *S. oryzae* (7-day-old) or *C. maculatus* (4-day-old) adults into glass jars (250 ml volume) filled with 100 g of rice or cowpea, respectively. The introduced adults were removed after 10 days. Each experiment consisted of seven treatments (1% and 5% *C. sophera* leaf powder, untreated control, *C. sophera* hot- and cold-water extracts (12.5% w/v), and hot- and cold-water controls). Each treatment was replicated 10

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