Bruchid control with traditionally used insecticidal plants *Hyptis spicigera* and *Cassia nigricans*

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Abstract

Losses of stored seed to insects in the tropics have reached levels of major concern. Synthetic insecticides while effective are invariably too expensive. The efficacy of two plants, *Hyptis spicigera* and *Cassia nigricans*, used by farmers to control insect infestation in stored cowpeas was determined. The oviposition and hatching of bean weevils (*Acanthoscelides obtectus*), under controlled environmental conditions, were reduced following treatment with EtOH extracts at low application rates with EC_{50} between 0.3-14 µl extract/g bean. Further field studies are proposed to determine if such natural products can be further exploited to reduce stored legume losses.

Introduction

Subsistence agriculture requires that the farmer store a percentage of staple food to feed his family and livestock between harvests. Giles (1964) has suggested that the largest quantity of food in the tropics is stored in the traditional farmer's small granaries. It has been an age old practice of traditional farmers in many regions of the tropics to mix a local plant (weed) with the grain because of some antifeedant/repellant component against stored grain pests.

In Upper Volta cowpeas may be stored in the pod in large wicker containers with a conical thatched lid. The open weave while not inhibiting insect movement does allow free movement of air. Mixed in with the pods are whole plants of *Hyptis spicigera* or leaves from *Cassia nigricans* as a means of controlling bruchid beetles (*Callosobruchus* sp.) whose larvae feed on the grain, causing extensive damage and loss. A second form of storage is a mud container into which podded cowpeas are placed along with ground-up *Hyptis* or *Cassia* and then sealed.

In recent years losses of stored seed to insects in the tropics have reached levels of major concern and a variety of methods of control including traditional methods have been investigated. Prevett (1961), in studying infestation of cowpeas in Nigeria by two species of bruchids, concluded that normal means of cultivation precluded control in the field at the oviposition peaks but a reduction of oviposition by treatment of stored cowpeas and treatment of pods during the immediate post-harvest storage period could reduce considerably levels of infestation. The insecticidal value of the essential oil of Acorus calamus has been examined against the pulse beetle Bruchus chinensis (Yadava, 1971). The product was found to be non-hazardous, did not impair seed germination and if used as a dressing on the seeds before storage ensured they would be free from pulse beetles for many months. Schoonhoven (1978) reported that vegetable oils at 1 ml/kg of seed provided a high level of control against the bruchid Zabrotes subfasciatus. Inspite of these encouraging results, the initial reaction from the developed countries has been to offer insecticides. Such synthetic products usually have a high potency, but are extremely expensive for the small farmer of the tropics and if not treated with deference can be toxic to the handler(s) and are known to have detrimental effects on the environment (Perkins, 1982).

The aim of the study was (1) to evaluate the efficacy of Hyptis spicigera and Cassia nigricans and (2) determine the effective levels of application (EC_{50}) of these plant materials necessary to afford satisfactory suppression of bruchid beetle activity under controlled environmental conditions. Extracts of the plants made with material collected in the field and immediately preserved in alcohol, were also used, in order to assess the presence of more labile insecticidal substances in the plants.

Materials and Methods

Maintenance of cultures:

The bean weevil *Acanthoscelides obtectus* was raised in Mason jars on a diet of white beans *Phaseolus vulgaris* maintained in an environmental chamber at 27°C, relative humidity (RH) 70% and photoperiod of L/D: 16/8. Under these conditions, emergence of new adults took place after approximately one month. Therefore the cultures were left undisturbed for this period after which time the dead adults were removed from the culture by sieving. The beans were transferred to a clean Mason jar to await eclosion of adults from pupae. Beans used as diet and for tests were kept in a cloth bag in the environmental chamber to ensure constant moisture content.

Preparation for tests:

According to Szentesi (1972) and Pankanin-Franczyk (1980), the peak of female egg laying occurs about 3-4 days after emergence begins. Therefore all insects used on tests were no older than 48 hours. Age of insects was ensured by removing all emerged adults from particular cultures by sieving and then using insects that emerged over the next two days. A series of soil sieves may be used to remove the insects and frass including a cover, 4.00 mm and 1.00 mm sieves and bottom pan. Insects not used on a test may be used to start a new culture so that cultures with emerging insects are available continuously. Insects were sexed by anesthetizing with CO_2 and examining the shape of the pygidium and slope of the abdomen under a dissecting microscope (Howe and Currie, 1964).

For all treatments with either solvent extracted or dry plant material, approximately 50 beans weighing 10.0 ± 0.1 gm were used. Tests were carried out with ethanol extracted Hyptis, and Cassia from Upper Volta, dry Hyptis and dry Cassia from Upper Volta, dry Hyptis from Sierra Leone, a water soluble and an ether soluble fraction of Sierra Leone Hyptis and a further four fractions of Upper Volta Hyptis in methanol. A volume of 300 µl of extract was found to be sufficient to cover completely 10.0 gm of beans and also dried fairly quickly. Three preference tests were also carried out using five different plant species extracted in ethanol including Hyptis from Upper Volta, Cassia, Khaya, Azadirachta and Cymnopogon.

Each solvent extract test consisted of five treatments of different volumes of stock plant extract (1 gm/ml) plus two controls, an ethanol control and a no treatment control. The beans (10.0 g) were treated with 3, 10, 30, 100 and 300 µl of plant extracts made up to a volume of 300 µl with EtOH. Treated beans were transferred to 25 x 95 mm shell vials for the tests.

Weights of dry plant material were 300 mg, 100, 30, 10 and 3 mg plus a no treatment control, using as before a 10 g bean sample.

Both preference and non-preference tests were carried out with the solvent extracted and dry Hyptis and Cassia and if possible each test replicated three times.

Preference tests:

Preference tests were carried out by arranging the shell vials in a circle equidistant from one another in a glass dessicator whose cover had had a hole cut in the knob on the lid. A plate was cut from cardboard to hold the vials upright which could rest on the plate rim on the inside of the dessicator. A piece of filter paper was also cut to the dimensions of the inside of the dessicator and cut to fit tightly around the vials so that insects could not escape into the bottom of the container. The plate-filter paper was taped around the edge for the same reason. Vials were placed randomly in the dessicator and the container covered. One hundred $\pm$ 1 insects, unsexed, were then introduced into the centre of the container through the hole in the lid which was stoppered with a foam plug.

Non-preference tests:

For non-preference tests, 20 insects of appropriate age, 10 $\pm$ 1 males and 10 $\pm$ 1 females, were introduced into each vial and the vial plugged with a foam plug.

All tests were kept in the environmental chamber under the same conditions as the cultures. Some tests were examined every two days for adult mortality and others left undisturbed for one month. After one month, before new emergence started, the number of eggs laid in each vial was counted. This was done by examining each bean under a dissecting microscope to count any eggs adhering to the beans and then gradually and carefully brushing the rest of the eggs out of the vial with a small paint brush. Counting was facilitated by brushing the eggs out onto a black cardboard plate with a numbered grid drawn on it. Tests were then returned to the chamber to wait for new emergence. Once emergence started the test was checked every two days and the number of adults emerged counted and removed from the vial. Adults removed were kept in a separate container for sexing later so the pattern of male and female emergence could be examined. An attempt was made to remove any eggs laid by offspring when the tests were checked every two days.

Results and Discussion

On all tests there was little difference between controls and experimental conditions in the time required to reach emergence. Male and female emergence followed a similar pattern with males generally emerging first. The majority of adults emerged over a 16 day period, which was also observed by Szentesi (1972). On preference tests examined for adult mortality, little difference in life span of adults between controls and treated beans was observed and in several instances, adults survived longer on treated beans.

Although all tests were not run concurrently results from similar tests have been plotted together to compare effectiveness of different plant extracts.

Increasing concentration of ethanol extracts of *Hyptis* (3-300 µl) resulted in a sharp decline (EC50 = 22 µl extract/g beans) in total numbers of eggs laid in three preference tests (Fig. 1). In two trials no eggs were laid on 300 µl concentrations and only 2 on the third trial. Percent emergence (number of adults emerged with respect to total eggs laid) was high (67-91%) on all three trials up to 30 µl and then reduced to an average of 8.0% from 100 µl treated beans. No emergence occured at 300 µl.

Egg laying declined on three preference tests with ethanol extracts of Cassia at concentrations above 30 µl (EC_{50} = 0.32 µl/g). No eggs were laid on two of three replicates on 300 µl and 900 µl treatments (Fig. 1). Zero emergence occurred at 900 µl and only one insect emerged from 300 µl treated peas.

While both species are effective in reducing total number of eggs laid Cassia appears more effective at the lower concentrations.

On non-preference tests using the same Hyptis extract the results were variable although again there was a general decline in oviposition on all three trials (EC_{50} = 7.9 µl/g) (Fig. 2). Eggs laid per female were reduced by an average of 61.7% with respect to ethanol controls and 30.7% with respect to untreated controls. It appeared that ethanol may have a stimulating effect on egg laying.

For the Cassia extract there was a consistent decline in eggs/female with increasing concentration (Fig. 2) (EC_{50} = 1.3 µl/g).

Few emergent adults were obtained from disturbed non-preference tests. We assume that the continuous disturbance had an adverse effect on eggs or nearly hatched larvae. No attempt was made to obtain emergence data from these non-preference trials because of the disturbance factor.

Three no choice tests with Hyptis extracts were repeated to obtain emergence data. Percent emergence was variable on all trials up to 30 µl. At higher concentrations, a sharp decline (EC_{50} = 14 µl/g) in percent emergence was evident, (Fig. 3). Zero emergence occurred in two of three trials at 300 µl. On one Cassia non-preference trial, emergence was reduced 47% at 90 µl and completely inhibited at 300 µl (Fig. 3) (EC_{50} = 3.6 µl/ml).

Dry powdered Hyptis from Sierra Leone did not reduce egg laying on either preference or non-preference tests. Oviposition was variable in two preference trials on beans with 3-100 mg Hyptis, the most being laid on 300 mg. Percent emergence declined as weight of Hyptis increased (Fig. 4) but not nearly as sharply as in trials with extracts of Hyptis (Fig. 3). Because a large number of eggs were laid on 300 mg treatments, the question is raised as to whether this reduction is partially due to density of eggs and not solely to dry Hyptis.

A single preference trial performed with dry Cassia from Upper Volta yielded a similar pattern of oviposition as dry Hyptis. A continuous decline (EC_{50} = 2.5 mg/g) in percent emergence from 30-300 mg Cassia occurred, resulting in a total reduction of 78% (Fig. 4).

Of three non-preference tests, one replicate was performed with dry Hyptis from Upper Volta and two replicates with dry Hyptis from Sierra Leone. Percent emergence was significantly reduced with increasing concentration for the Upper Volta sample (EC_{50} = 7.1 mg/g), but not for the Sierra Leone samples (Fig.

5). Possible reasons for the difference in emergence could be due to a varietal difference in Hyptis and/or because the Sierra Leone sample was collected six weeks after the Upper Volta sample. If the compound was volatile we would expect that it would lose its repellant value as the plant, an annual, completes its life cycle.

Oviposition was variable on three non-preference replicates with Cassia, one showing steady decline in eggs/female, one showing an increase in eggs/female with increasing Cassia and the other an initial increase followed by a decrease at 100, 300 mg. Percent emergence declined (EC_{50} = 12.6 mg/g) on all three replicates from 30 mg Cassia (Fig. 6). Average reduction in survival of progeny at 300 mg was 91.8%.

Ethanol extracts (30 µl/10 g beans) of Upper Volta Hyptis, Cassia, Azadirachta indica, Cymnopogon and Khaya were used together on three preference tests. Oviposition and percent emergence data indicated that Cymnopogon was the most repellant species followed by Azadirachta, Cassia, Hyptis and lastly Khaya.

Conclusions

EtOH extracts (1 g/ml) of Hyptis and Cassia from Upper Volta both reduced oviposition and emergence of A. obtectus at low application rates with EC_{50} between 0.3-14 µl extract/g bean. Although neither Upper Volta/Sierra Leone dry Hyptis nor dry Cassia deterred egg laying, a reduction in emergence was obtained using dry Hyptis and Cassia from Upper Volta but not dry Hyptis from Sierra Leone. A possible reason for the difference in efficacy is that the compound(s) in *Hyptis* may be volatile and/or labile and that the species and time and place of harvest may be important in its repellant value.

Detailed information on the traditional use of these plants by farmers is now necessary. Field efficacy trials at time of harvest coupled with isolation, identification and analyses of the levels of active principals from maturing *Hyptis* and *Cassia* would shed light on the longevity and possible volatility of the compounds. Under controlled environmental conditions significant reductions in emergence were obtained. It is now necessary to determine if such naturally occurring plant compounds can be exploited as a cheap means of reducing stored legume losses.

Acknowledgement

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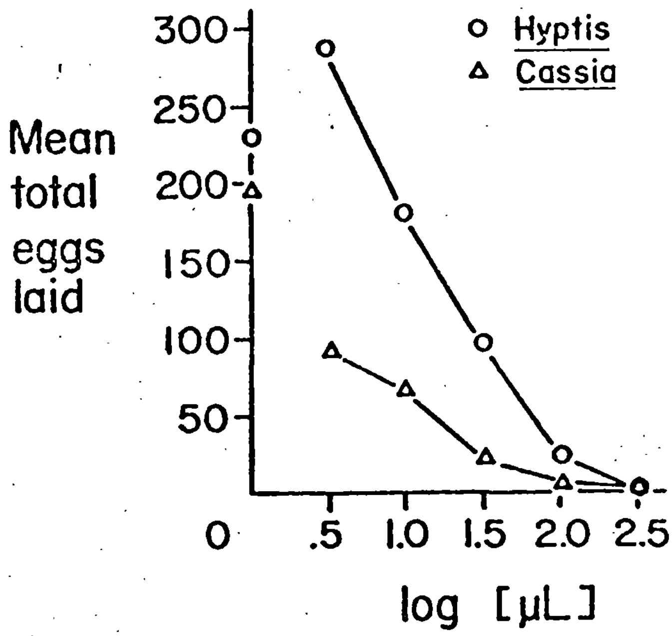

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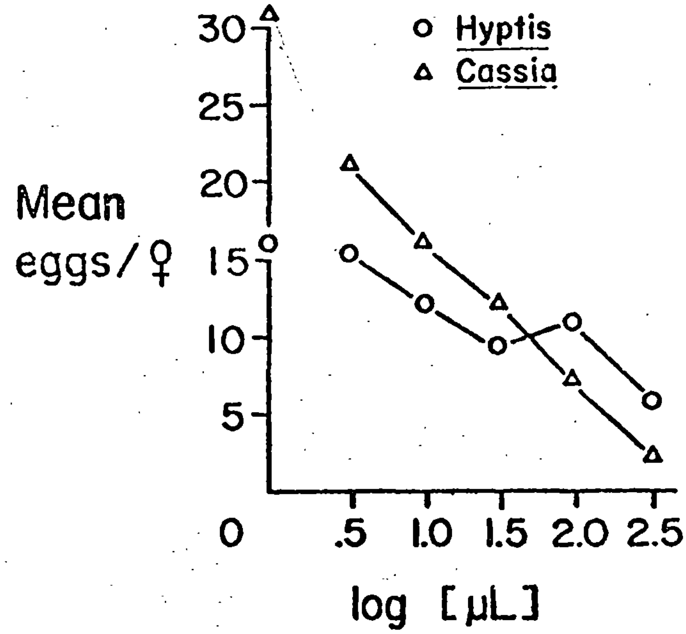

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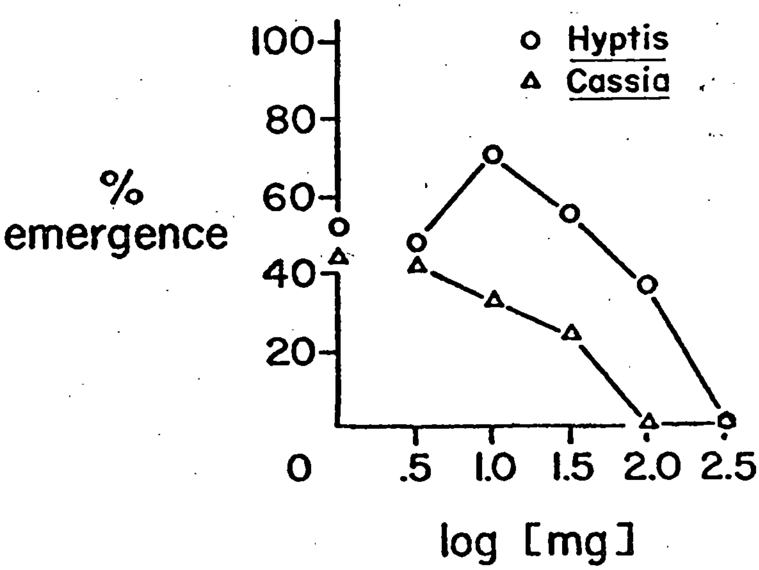

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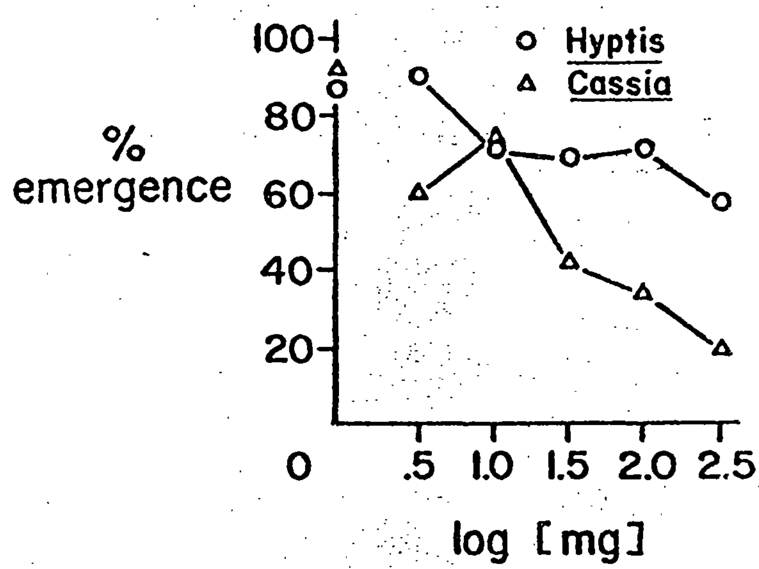

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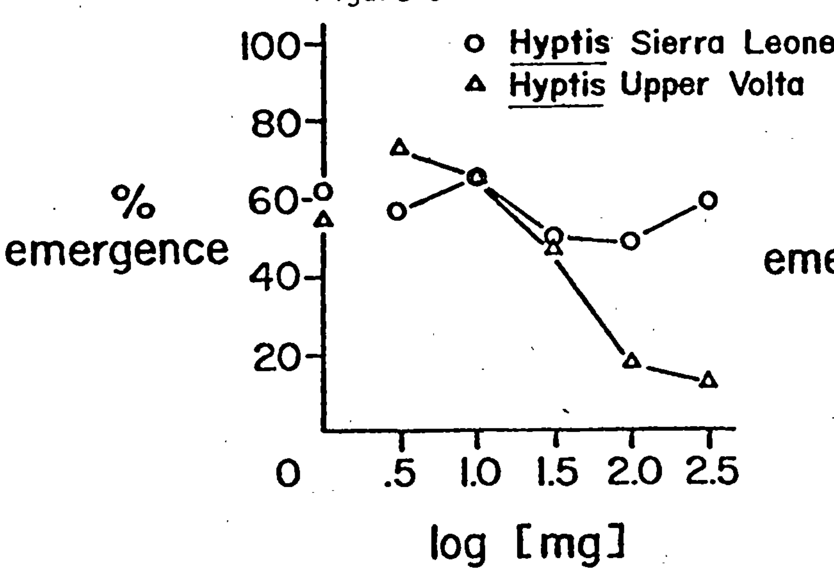

Figure 6