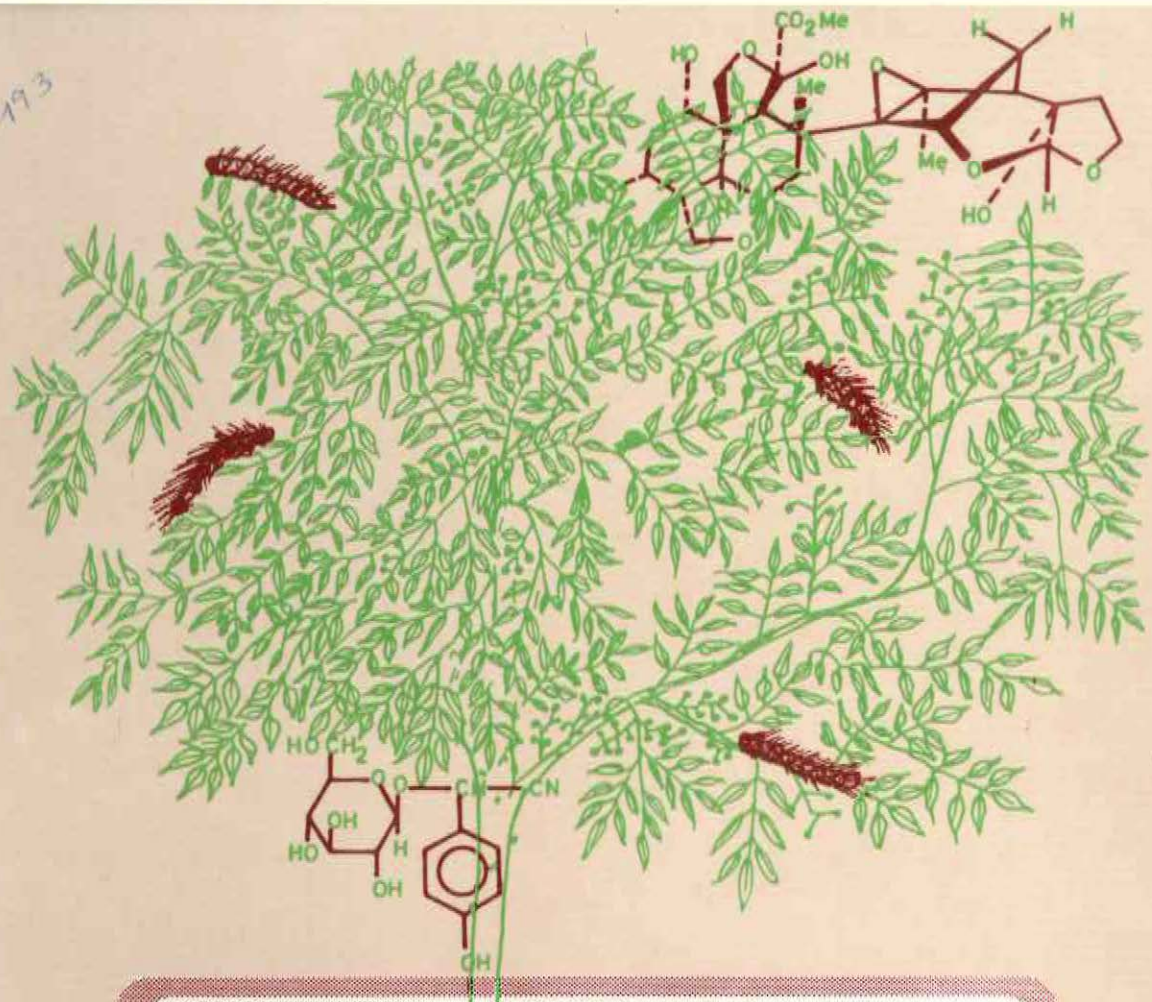

BIOPESTICIDES AND PEST MANAGEMENT IN THE DEVELOPING WORLD

PROCEEDINGS

An International Study Workshop on Newer Methods in the Isolation, Characterization and Evaluation of Biopesticides

International Centre of Insect Physiology and Ecology (ICIPE)
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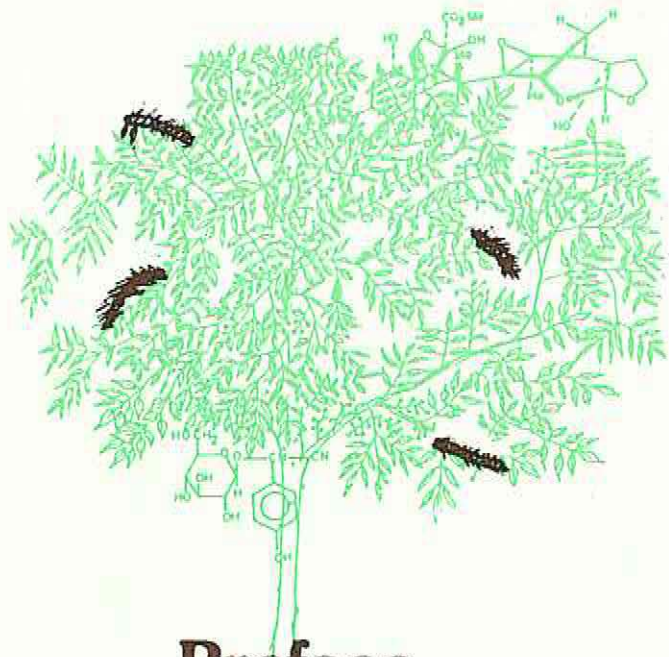


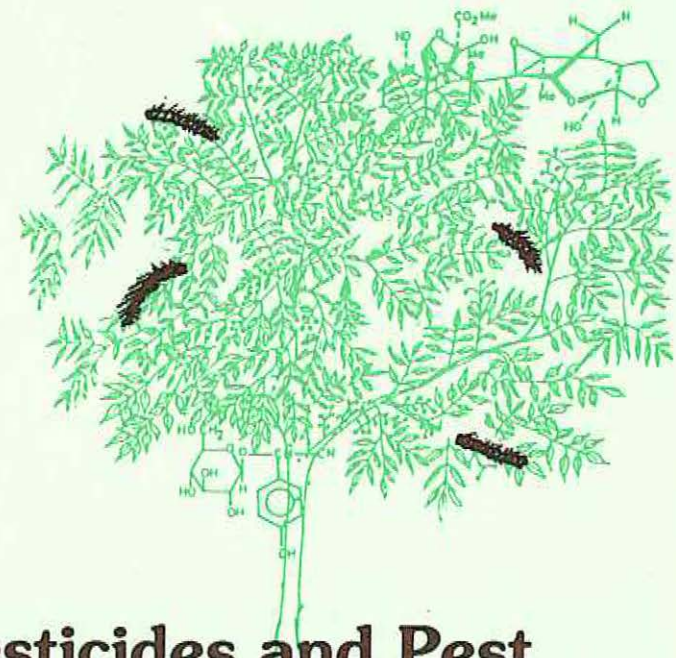
Preface

Preface

"The International Study Workshop on Newer Methods in the Isolation, Characterization and Evaluation of Biopesticides", held in Nairobi (Jacaranda Hotel and ICIPE), 16–25 April 1990, was sponsored by the United States Agency for International Development (USAID) and organized jointly by the Board on Science and Technology for International Development (BOSTID) of the United States National Research Council and the International Centre of Insect Physiology and Ecology (ICIPE).

The purpose of the Workshop was to bring together scientists working in the broad area of biopesticides (natural products, pheromones, biocontrol agents, etc.), in order to identify constraints in their research work and to explore ways of sharing experience, information and facilities, through collaboration and networking. Although the Workshop focused mainly on the work of scientists from Africa that of scientists from elsewhere (Asia and North America) was also presented and discussed.

The present publication has arisen from the papers presented in that Workshop and includes a distillation of major observations and recommendations made in a lively discussion that took place in a special session devoted to reflect on the promise that biopesticides held for Africa and on ways of realizing that promise. We hope that it represents both a record of the proceedings of that workshop and a reminder of what needs to be done in this important area.



Biopesticides and Pest Management in the Future

1

Paul B. Capstick

Biopesticides and Pest Management in the Future

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With the tremendous growth in the world's population, major challenges will have to be faced in the coming decades. Concerning food supply, the agricultural self-sufficiency of a single country is not the main issue, rather the global food supply.

Problems with overproduction in some countries and famine with others, difficulties with the transfer of technology between them and growing ecological concerns in a world with limited resources sometimes combine to encourage scepticism towards research, and doubts as to whether science will be able to solve future problems (Brundtland Report, 1987). It would be unfortunate if this trend should lead to the reduction in efforts being made to assure a better food supply in the future. Pest management, is an important aspect of these efforts.

Pest and weed control are not an end in themselves but a way of protecting the plant, thereby ensuring good yield and high quality. It is now generally accepted within the international scientific community that complete eradication of diseases, pests or competing weeds is not possible and, apart from ecological consequences of such drastic action, is also not necessary because to a certain extent the crop plant and animal host can depend on its own competitiveness and natural defence mechanisms. It is only when damage surpasses economic thresholds do crop protection and animal treatment measures become necessary.

Pest management means the sum total of all efforts to keep pests, diseases, and weeds below this damage level with suitable products or methods, using efficient application techniques and the right timing, with the necessary followup, and by maintaining a balance between the economy and the ecology. The success of such an approach will, in part, depend on whether plant and animal production can keep pace with the growing world population.

Twenty-three years ago, Cramer (1967) estimated that losses in plant production up to harvest caused by pests, diseases and weeds totalled one-third of the potential yield. More recent estimates (Ahrens *et al.*, 1984) have indicated a similar global situation. In Western Europe, with its intensive crop protection practices, the loss is sometimes much lower, mainly in those areas where, for example, under favourable conditions 10 tons per hectare of wheat can be harvested today. On the other hand, insufficient or inadequate crop protection in developing countries cannot always prevent a total loss.

The low intensity of current production in some developing countries suggests considerable potential for yield increases under locally appropriate conditions of pest management and agricultural practices, for example the average wheat yield in Western Europe is 3.67 tons per hectare, as compared to 1.53 tons per hectare in developing countries (Kraus, 1985).

For almost half a century man has depended on pesticides to control the insects, diseases and weeds. Indeed when the 'Age of Pesticides' arrived with the commercial introduction of DDT in 1946 (Metcalf, 1980) it seemed that the possibility of controlling insect pests for ever, indeed their eradication, appeared a distinct possibility — and the 'Era of Optimism' (1946–1962) was ushered in. The synthetic pesticide model of DDT brought in a whole array of synthetic pesticides in quick succession. It seemed then that pests as a whole were destined to oblivion within a matter of time. Thus, in the USA, three large commercial crops (cotton, maize and apple) between them utilized 67% of all farm-applied insecticides (Metcalf, 1980). Yet, over the 75 years since 1900 that the United States Department of Agriculture (USDA) has kept records, there is no evidence that crop damage from insect infestation has decreased: indeed, if anything, these losses have increased steadily for cotton and maize and there has not been much advance in mitigating losses in apple.

Furthermore, if one closely examines the quantity of insecticides applied as against the level of resulting crop production, one is startled to find that there is a distinct decrease in the production of maize per lb of insecticides employed, from a ratio of 29,160 bushels of grain/lb of insecticides in 1946 down to a ratio of 207 bushels/lb in 1971. Similarly, for cotton, the ratio has decreased from a production of 3.80 bales of cotton for each lb of insecticides applied in 1919 down to a ratio of 0.142 bales/lb in 1971.

The marked decline in the responsiveness of plant pathogens and insects to pesticides in the world is due to several important factors, which include the following: increasing pesticide resistance of pathogens and insects; the destruction of natural enemies of certain pests (and, therefore, the need for additional pesticide application); reduced crop rotations and crop diversity, with increased reliance on continuous culture of monocrops; the planting of crop varieties, although high-yield, which are susceptible to pests; and increased 'cosmetic standards' (Pimentel, 1978). Similar factors are already operating in the tropics, to which must be added the problem of resurgence of pests following repeated insecticide applications.

It is precisely because of these inherent drawbacks of the broad-spectrum pesticides that have precipitated an urgent need to find newer biopesticides.

REQUIREMENTS AND REALIZATION OF NEWER BIOPESTICIDES

Newer biopesticides must be adaptable to the needs resulting from the interaction between the èconomy and the ecology.

Today's requirements result from the experience and success achieved so far, which can also be projected into the future. A biopesticide will only be successful on the market when it provides advantages over existing compounds. Conditions for the successful development of new biopesticides can be illustrated as follows:

Rate: While older broad-spectrum pesticides were known to be active in the kilogram range, the newer biopesticides must be effective in the gram range.

Formulation: Biopesticides have to be formulated in such a way that the active ingredient can be distributed as evenly as possible. Formulation technology should also improve the handling and application of the biopesticide. Liquid formulations without organic solvents and dry formulations with decreased dusting have been shown to provide better safety.

Activity spectrum: While in the past a farmer could use only broad active pesticides, he now needs a wide range of choice from which he can select a specific compound or a combination product which exactly suits his situation.

Mode of action: Special properties of a bioinsecticide will allow greater flexibility in pest management. The systemic property, for example, provides protection even after penetration of the pest into the plant tissue. Selective herbicides can also be applied even after emergence of the crop plant.

Environmental research: Only bioinsecticides which pass strict toxicological tests, show no accumulation in the soil and cause no groundwater contamination through leaching will be chosen for further development. The protection of beneficial organisms is an additional criterion which will become increasingly important in the future.

The final results of all these research efforts are crop and animal protection products which meet today's requirements of efficacy, safety, economy and ecology. These criteria will also have to be met by tomorrow's biopesticides, whether based on conventional chemical synthesis or biotechnology. Therefore, development of a new biopesticide will take something like 8 to 10 years and cost in excess of US $50 million. Only major crop pest and animal health protection problems and world markets can justify these efforts and costs. The consequence of such an internationalization of crop and animal protection would be a concentration of the biopesticide producing industry. Only those companies which have their own broad research and development facilities and the necessary financial support for long term operations will be able to come out with a wide range of products.

In contrast to the crop and animal protection industry, which is becoming more and more international, pest management has no uniform global dimension. It is greatly influenced by regional aspects which lead to differing concepts of agriculture and crop protection. In developed countries emphasis is no longer placed on enhanced yield, but on stabilizing the yield and quality. In contrast, improvement in food supply is still the driving force in developing countries, with great emphasis placed on pest management.

MANAGEMENT OF R&D FOR BIOPESTICIDE PRODUCTION

Biopesticides can only be produced successfully if R&D efforts are managed accordingly. All the activities necessary for the development of new biopesticides have to be well co-ordinated in an elaborate time-frame. The cost of environmental research (toxicology, ecobiology) has increased from less than one-fifth of the total R&D costs in 1975 to an expected one-third in 1988.

The crop protection and animal health industry regards this effort as necessary for the safety of the producer, the applicator, the consumer and the environment, but it has to be stressed that registration of the biopesticides has to be managed in order to standardize and harmonize the requirements. When national or even state regulations differ so greatly that the R&D efforts have to be multiplied, fewer and fewer companies are able to afford expenditure.

Nevertheless, the prospects for the development of long-term and sustainable biopesticides are good and we should make a firm decision to invest mission-oriented R&D work which would attain this goal in the very near future.

It is for this reason that it is most gratifying that this International Study Workshop brings together an array of talent from industry, academic and government agencies from different countries to discuss and identify new opportunities for newer methods in the evaluation and characterization of biopesticides.

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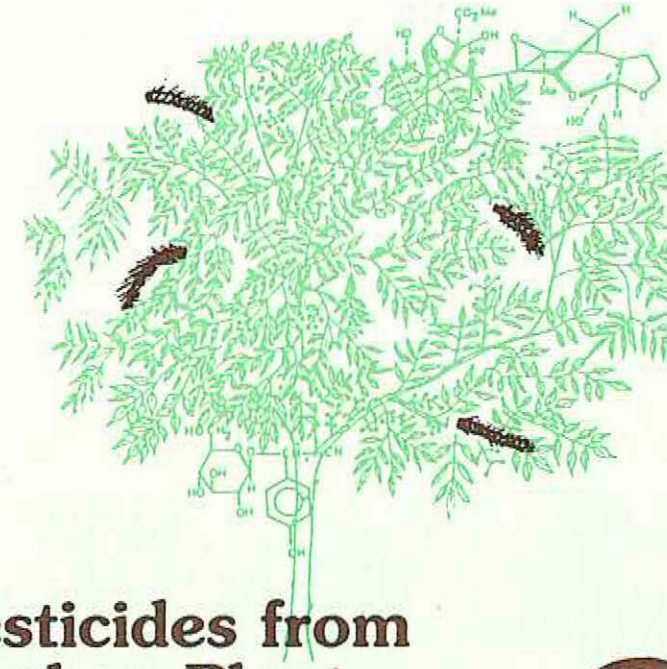

Biopesticides from Sri Lankan Plants

2

Vijaya Kumar

Biopesticides from Sri Lankan Plants

Vijaya Kumar
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Sri Lanka has a flora of about 3500 plant species, a quarter of which are endemic to the country. This flora occurs in a wide range of environments — dry zone, coastal regions, rain forest and high altitude forests — within the small land mass of the island. Since many of these plants, especially those peculiar to the country had never been examined for insecticidal activity, a programme was begun of screening plant extracts for insecticidal, fungicidal, nematocidal, acaricidal, parasiticidal and herbicidal activity. The screening was carried out at Basle, Switzerland by Ciba-Geigy Ltd., who funded the programme.

During a seven year period, about 600 plant extracts were screened under the programme and many of them found active (Table 1). The plants themselves were chosen on the basis of Sri Lankan folklore on plant use in pest control, field observations on their resistance to attack in their natural environment and relationship to plants reported in the literature to show activity. The plants were sequentially extracted with dichloromethane and methanol to obtain the extracts for screening.

Plants screened from 24 families did not show any activities (Table 1). Although in most cases the number of extracts screened was insufficient to generalize on family characteristics, it was apparent that members of the families Apocyanaceae, Logianaceae, Moraceae and Symplocaceae showed little activity. Even among the Sapindaceae, only weak activity was shown by a few extracts, while only one member of the Celastraceae showed any activity at all.

Some families like the Meliaceae, Lauraceae and Rutaceae showed a high percentage of positive results (Table 2). Members of the Meliaceae and Rutaceae families showed a broad range of activities, unlike the Lauraceae which showed mainly nematocidal and acaricidal activity. A fair proportion of the Tiliaceae too showed nematocidal and acaricidal activity. It is not surprising that phytotoxicity is found mainly in root extracts. Herbicidal activity of roots can provide plants with an effective weapon in the struggle against competing plants.

Table 1. Plant families showing activity

Family	Number of extracts			
	Screened	Inactive	Active*	Weakly Active
Alangiaceae	2	2	–	–
Amaryllidaceae	6	3	2	1
Anacardiaceae	12	9	–	3
Annonaceae	5	3	2	–
Apocyanaceae	10	10	–	–
Araceae	2	1	1	–
Araliaceae	2	2	–	–
Asclepiadaceae	4	4	–	–
Bignoniaceae	2	2	–	–
Bombacaceae	10	8	2	–
Burseraceae	2	2	–	–
Celastraceae	20	19	1	–
Clusiaceae	10	6	3	1
Combretaceae	6	4	2	–
Compositae	2	2	–	–
Convulvulaceae	2	1	1	–
Dilleniaceae	4	4	–	–
Dipterocarpaceae	13	11	2	–
Erythroxylaceae	4	4	–	–
Elaeocarpaceae	4	4	–	–
Euphorbiaceae	10	7	2	1
Fabaceae	20	16	4	–
Gramineae	4	3	1	–
Hippocarateaceae	2	2	–	–
Iridaceae	2	2	–	–
Lauraceae	21	13	7	1
Lecythidaceae	10	6	3	1
Longaniaceae	8	8	–	–
Lythraceae	4	4	–	–
Magnoliaceae	14	12	1	1
Malvaceae	10	8	2	0
Meliaceae	41	25	15	1
Menispermaceae	8	7	1	–
Mimosaceae	6	4	2	–
Monimiaceae	2	2	–	–
Moraceae	17	17	–	–
Myristicaceae	6	5	–	1
Myrsinaceae	4	3	1	–
Myrtaceae	4	4	–	–
Opiliaceae	2	2	–	–
Pandanaceae	2	2	–	–
Pittosporaceae	4	4	–	–
Polygalaceae	2	2	–	–
Proteaceae	8	7	1	–
Rhizophoraceae	4	4	–	–
Rubiaceae	2	2	–	–
Rutaceae	59	41	14	2
Salvadoraceae	2	1	1	–
Santalaceae	8	6	–	2
Sapindaceae	27	21	–	6
Sapotaceae	22	19	3	–
Schisandraceae	2	–	1	1
Staphyleaceae	6	6	–	–
Symplocaceae	8	8	–	–
Theaceae	10	8	–	2
Thymelaeceae	4	3	1	–
Tiliaceae	11	6	3	–
Verbenaceae	12	8	2	2
Zingiberaceae	4	3	1	–

*Strong or moderate activity.

Table 2. Types of activity shown*

Family	Insect	Animal parasite	Fungi	Nematodes	Acarus	Phyto-toxicity
Amaryllidaceae	–	2Rt	–	–	–	–
Annonanceae	–	1Sb	1L	1L	1Sb	–
Araceae	1Sb	1Sb	–	1Sb	–	–
Bombacaceae	–	–	–	–	1Rt	1Rt
Celastraceae	1Sb	–	–	–	–	–
Clusiaceae	2Sb	1Sb	–	1Sb	–	–
Combretaceae	IL	–	–	1Sb	–	–
Convulvulaceae	IL	–	–	–	–	–
Dipterocarpaceae	2L	IL	–	–	–	–
Euphorbiaceae	–	–	–	–	2L	–
Fabaceae	1Rt/IL	2L	–	1Rt	1Rt/1L	–
Gramineae	1Rt	–	–	–	–	1Rt
Lauraceae	1L	–	–	IL	2Sb/2L/2Rt	–
Lecythidaceae	1Fr	1Fr	–	–	1Sb	–
Magnoliaceae	1Rt	–	–	1Rt	–	1Rt
Malvaceae	1Fr	–	–	–	–	1Rt/Fr
Meliaceae	4Sb/2Rt/1L/1Fl	1Sb/1L/1Fl	1Sb	1Sb	–	2Sb/ 2Rt/1Fl
Menispermaceae	1Sb	–	–	–	–	–
Mimosaceae	–	–	–	1Rt	–	1L
Proteaceae	–	–	–	–	1Sb	–
Rutaceae	4Sb/3Rt/2L	1Sb/1Rt/2L	1Rt	2Sb/1Rt/2L	–	1Sb/2Rt
Salvadoraceae	1L	1L	–	–	–	–
Sapotaceae	1L	–	1Sb/1L	–	–	–
Schisandraceae	–	–	–	–	–	1Rt
Thymeliaceae	1Sb	–	–	–	1Sb	–
Tiliaceae	–	–	–	1Fr	1Rt/1L	–
Verbenaceae	–	–	–	1Rt	1Rt	–
Zingiberaceae	–	–	–	–	–	1Rh

*No. of extracts of particular plant (Sb—Stem bark, Rt—Root, L—Leaves and twigs, Fr—Fruits, Fl—Flower, Rh—Rhizome) showing strong or moderate activity of type specified.

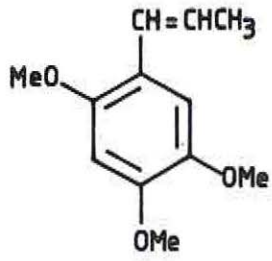
The best results obtained so far have been with extracts from two Sri Lankan plants that showed herbicidal and fungicidal activity at commercially interesting levels.

One of the problems faced in carrying out screening abroad was time. It took about 6 months for screening results to be reported. Isolation of an active pure compound through enrichment of active material by repeated fractionation followed by screening of fractions could take several years. The screening programme abroad was also limited to activities of interest to our collaborators and did not cover those areas in Sri Lanka considered important, e.g. tea and coconut pests. Therefore, a screening programme was started in Sri Lanka about 5 years ago. It began by screening extracts and pure compounds for fungicidal activity against *Cladosporium cladosporioides* using a TLC-bioassay (Klarman and Stanford, 1968). Although several compounds isolated showed activity in this *in vitro* bioassay, it was disappointing

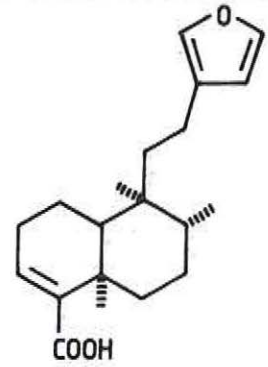
to note that none of these were reflected in the results of *in vivo* screening at Basle against agriculturally important fungi. This underlined the need to use *in vivo* bioassays wherever possible in activity studies.

Routine insecticidal screening was begun with activity against the groundnut aphid, *Aphis craccivora*, which is a serious pest on cowpea and other legumes in Sri Lanka. The aphid was cultured on cowpea seedlings. One-day old apterous female adults on cowpea leaves were sprayed with homogenized extract suspensions at concentrations of 4000 ppm. Commercial dimethoate was used as the standard insecticide. Where significant activity was observed, the effect of the extract on larval stages and its mode of action were investigated. The extract was then fractionated and an attempt made to isolate the active fraction by enrichment.

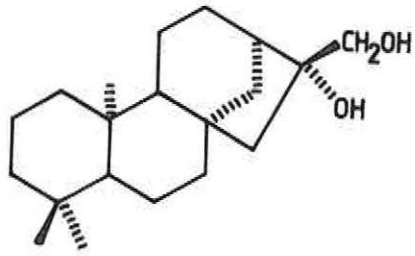
About 50 plant extracts being studied at Peradeniya were screened, 10 extracts were found to have significant activity. Extracts for *Acorus calamus* and *Costus speciosus* rhizomes, *Pleiospermum alatum* stem bark, *Croton lacciferus* and *Ocimum gratissimum* roots and *Celtis cannamomea*



(a) Asarone



(b) (-) - Hardwickiic acid[3]



(c) *ent*-Kaur-15-ene-3β, 17-diol[4]

Figure 1

leaves showed more than 80% mortality, 24 hours after treatment. *A. calamus* rhizome extract was shown to act through fumigant activity and by reducing the fecundity of adults produced when applied on 1st instar nymphs (Bandara and Peries, 1990). Its activity was shown to be due to β-asarone (Fig. 1a), which has previously been shown to be active against other insects. Some diterpenoid constituents of *Croton* species including (-) - hardwickiic acid (Bandara and Wimalasiri, 1985) (Fig. 1b) and *ent*-kaur-15-ene-3β, 17-diol (Bandara and Wimalasiri, 1988) (Fig. 1c) were also found to have aphidicidal activities.

Screening programmes to test for activity against the brown planthopper of rice, *Nilaparvata lugens* and the coconut caterpillar, *Opisinia arenosella* were initiated last year but so far none of the extracts tested have shown any significant activity.

ACKNOWLEDGEMENTS

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Natural Insecticides for *Spodoptera litura*

3

Pichaet Wiriyachitra

Natural Insecticides for *Spodoptera litura*

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INTRODUCTION

For self-defence, it is not surprising that plants would contain substances which are insect antifeedants, insect repellents and insecticides. Some naturally occurring antifeedants which have been published are: glycosides of steroidal alkaloids, demissine (Kuhn and Guhe, 1947); solacauline (Schreiber, 1957); tomatine (Buhr *et al.*, 1958); leptines I and II (Kuhn and Low, 1961); ring A enone and/or ring D aromatic steroids, the nicandrenones and others (Bates and Eckert, 1972; Begley *et al.*, 1972a; Begley *et al.*, 1972b; Gilbert and Norris, 1968); juglone (5-hydroxynapthoquinone) (Gilbert and Norris, 1968); the isoquinoline alkaloid isoboldine (Wada and Manakata, 1968); phenylpropanoids (Isogai *et al.*, 1973); germacranes sesquiterpenes shiromodiol and shiromol (Wada *et al.*, 1970a; 1970b); *ent*-clerodane and clerodane diterpenes, clerodendrin (Kato *et al.*, 1971); caryoptin and others (Hosozawa *et al.*, 1974); the hydroxylated steroid meliantriol (Lavie *et al.*, 1967); azadirachtin (Zanno *et al.*, 1975); xylomolin (Kubo *et al.*, 1967); ajugarins (Kubo *et al.*, 1976a); harrisonin (Kubo *et al.*, 1976b); polygodial ugandensidial and warburganal (Kubo *et al.*, 1976c); inflexin (Kubo *et al.*, 1977); isodomedin (Hedin, 1977a); crotepoxide (Hedin, 1977a) and unedoside (Hedin, 1977b). However there is no report of any of these substances being used effectively in agriculture.

A number of naturally occurring compounds have been found to exhibit insecticidal activity. These include alkaloids, terpenoids, phenols, amines, chromenes, prenylated guinones and sesquiterpenes esterified with phenolic acids (Burkill, 1966). Commercially, the substances which have found their way to agricultural application are nicotine, derris, pyrethrum and the newer product, ryania (Grainage *et al.*, 1985). Crude extracts of the plant producing these substances, or dried and powdered plant tissue have a long history of use in SE Asia as insecticides. However, none of these natural products is a universal insecticide: Tobacco leaf containing nicotine is subject to attack by several insects, the root of derris-containing plant is attacked by the larvae of a species of beetle, the flower from which pyrethrins are extracted needs synthetic insecticides for protection from insects in the fields. In addition, insects can develop resistance to a certain chemical

after a long exposure. New and better insecticides will, therefore, have to be constantly sought.

Since *S. litura*, is one of the major pests in SE Asia, the discovery of a new and more effective insecticide with low mammalian toxicity from local plants would produce enormous benefit to the region as well as to Thailand in general.

Spodoptera litura Fabr. is a widespread insect pest. It is polyphagous and regarded as one of the most damaging pests in the world agriculture. It was reported that the insect attacks 112 species of cultivated crops all over the world. In Thailand, at least 30 varieties of plants are found to be host plants of this insect (Tikwattananont, 1977). These include vegetables, floral plants, ornamental plants, economic field crops and weeds.

The time required for egg, larval and pupal development are 2–3, 14 and 7–8 days, respectively (Patoomchartipat, 1977). The adult longevity is 8–10 days in the male and 10–12 days in the female. The larva moults five times. It is at this stage that the insect is most damaging. Since the early larval instars are quite vulnerable to external damage, it has been chosen as the target organism for this work.

OBJECTIVES

1. To isolate and identify natural insecticides and anti-feedants, which have low mammalian toxicity for *S. litura*.
2. Extract or synthesize those compounds on a practical scale and design of industrial extraction procedure.
3. Evaluation of their applicability in the field.

MATERIALS AND METHODS

Plants investigated were collected in the southern region of Thailand. Samples were classified taxonomically and vouchers were deposited in the herbarium in the Department of Biology, Prince of Songkla University.

Ground plant materials were steam distilled or exhaustively extracted with solvents. The extracts/steam distillates were screened for insecticidal and anti-feedant activity with *S. litura* and the active extracts resolved into fractions. Each fraction was screened for activity and the active fraction, again, separated into individual constituents using quick column and preparative layer chromatography on silica gel.

Each of the individual components was tested again for activity and the LC_{50} value of the active compound determined. The structure of the compound was elucidated using spectroscopic techniques.

The leaf dipping (LD) method was used for the detection of stomach poisoning and anti-feeding activity; the third instars of *Spodoptera litura* larvae were left to feed on the leaves of *Vigna radiata* which had been immersed in the tested solution then air-dried. Ten larvae were used in each of the four replications (one as control). The number of dead organisms was recorded at the end of each day. The amount of the leaf consumption and the larvae size were also estimated.

The topical application (TA) method was used for the detection of dermal toxicity, the test solution was dropped on the thorax of the third instar larvae. The organisms were then left to feed on untreated leaves of *Vigna radiata* in a plastic container. Ten larvae were used in each of the four replications (one as control). The number of dead organisms was recorded at the end of each day.

The LD coupled with TA method was used for the detection of the combined effect of stomach poisoning and dermal toxicity. The test solution was dropped on the thorax of the third instar larvae. The organisms were then left to feed on the leaves of *V. radiata* which had been immersed in the same test solution then air-dried. Ten larvae were used in each of the four replications (one as control). The number of dead organisms was recorded at the end of each day.

RESULTS AND DISCUSSION

The workplan for this investigation was of three stages. Firstly, plants which are reputed to have insecticidal and antifeeding property were collected and screened for their activity. Secondly, the promising plant species were investigated for their active ingredients and the activity of these ingredients measured. Thirdly, field tests were carried on the appropriate formulations derived from the 'best' extracts/substances.

Over 150 plant species were collected and classified. Herbarium specimens were deposited either at the Prince of Songkla University Herbarium or Chiang Mai's Faculty of Pharmacy Herbarium. They were extracted either with alcohol to give crude alcoholic extracts or with a series of solvent with increasing polarity e.g. hexane, dichloromethane, methanol. The investigation further carried out on the interesting species are now summarized.

Zingiber purpureum

Systematic screening indicated that the active ingredients are a series of non-polar compounds which are steam volatile. Attempt to identify these compounds in the steam distillate from this plant revealed that they are a mixture of phenylbutanoids, in agreement with previous reports (Tuntiwachwuttikul *et al.*, 1981; Amatayakul *et al.*, 1979). The most active component appeared to be 4 (3', 4'-dimethoxyphenyl) but -1, 3 diene (Fig. 1). The compound was then synthesized, using the method

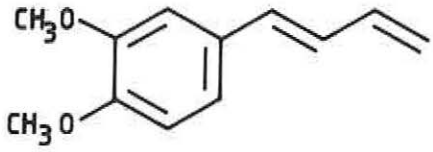

Figure 1

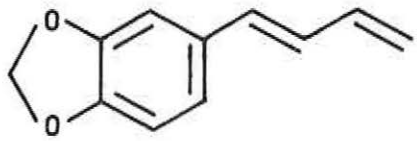

Figure 2

previously described (Amatayakul *et al.*, 1979), and then tested. LC_{50} for this compound was found to be 1.27% (48 hr), 0.52% (72 hr), 0.40% (96 hr), and 0.24% (120 hr).

In the study on the activity of pyrethrins, it was found that compounds containing a methylenedioxyphenyl group can act as synergists for the pyrethrins (Matsui and Yamamoto, 1971). The compound 4-(3', 4'-methylenedioxyphenyl) but -1, 3-diene (Fig. 2) was then synthesized, from sesamol with the same reaction scheme, and tested for activity, both alone and in combination with compound(1). While no synergism was observed, the activity of compound (2) was found to be higher than that of (1). LC_{50} of the compound was found to be 0.45% (48 hr), 0.23% (72 hr), 0.20% (96 hr), 0.18% (120 hr); and the LD_{50} value was 3.04 µg/insect (24 hr) in the TA test.

Ageratum conyzoides

The steam distillate of this common weed was found to be the active fraction. Isolation of the active substances gave precocene I (3) and

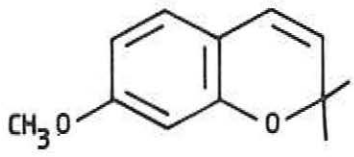

Figure 3

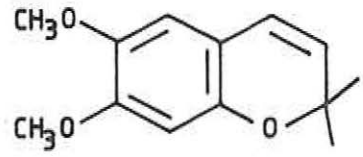

Figure 4

precocene II (4), which were previously found to be antijuvenile hormones of considerable interest (Bowers, 1983). The LC_{50} (TD) for the crude steam distillates, precocene I and precocene II are shown in Table 1.

Table 1. The LC_{50} values for the crude steam distillates, precocene I and precocene II

Material	48 hr	72 hr	96 hr
Distillate	0.83	0.37	0.29
Precocene I	0.66	0.29	0.11
Precocene II	0.84	0.71	0.35

Zingiber zerumbet

The active ingredients in the rhizome of this common zingiberraceous plant was found to be steam volatile and zerumbone (5) was isolated as the most active component in the mixture. The LD_{50} was found to be 527 µg/insect (24 hr) in the TA test.

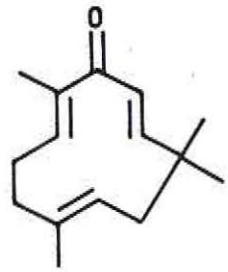

Figure 5

Field trial was carried out with various formulations derived from *Z. zerumbet* rhizome. The results, however, were not sufficiently satisfactory.

'Kaidum'

This plant species is used in Thai medicine. The specimen investigated was obtained from the Bangkok's Wat Mahadhat herbal market. Up to the time of this report, flowering has not occurred from the plant cultivated from the purchased rhizomes. The identity was therefore uncertain, although it was believed to be a member of the zingiberraceous family, known to the Thais as 'Kaidum'.

Test results showed that the steam distillates from the rhizomes contained the activity. The active principle isolated was identified as germacrone (6) and the LD_{50} value was similar to that of zerumbone.

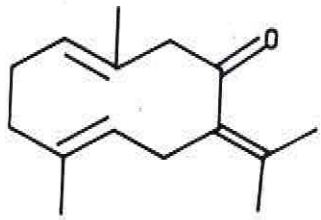

Figure 6

Aglaia odorata

Five species of *Aglaia* — *A. odorata, A. odoratissima, A. argentea, A. kunstlerii* and *A. squamulosa* — were extensively tested for activity and the best anti-feeding result was obtained from *A. odorata.*

From an active fraction, odorine (7) was isolated. This compound was, however, later found to be inactive. Further attempt resulted in an active substance which appeared to be one component on thin layer chromatography. This substance, from its spectroscopic evidences, was identified as scopoletin (8).

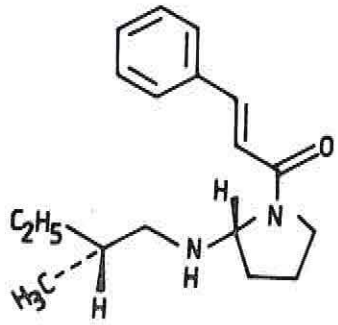

Figure 7

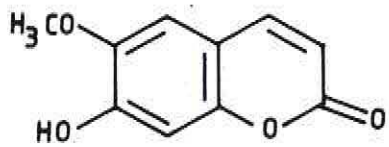

Figure 8

Small field trials were conducted on *V. radiata* with the methanolic extract and the aqueous extract of *A. odorata,* from which satisfactory levels of control, 81.3 and 75.5% respectively, were obtained. Similar

experiments on broccoli also gave satisfactory results. Trial on broccoli in farmer plots, however, yielded less satisfactory results. This was attributed to the inefficient method of spraying the formulations which did not reach the 'under' side of plant leaves where the insect larvae usually hide. It is believed that with a better spraying equipment which will deposit the formulation evenly on the plant leaves, this could be a formulation of choice for the control of *S. litura*.

Upon repeating the tests on scopoletin in order to obtain an LC_{50} value, it was found that an authentic sample of scopoletin did not give any activity. It was then understood that the sample of scopoletin previously tested must contain a very small amount of another component which was a very active anti-feedant. After several attempts, a very small amount of this substance was isolated. Spectroscopic evidence showed that this compound was rocaglamide (9). The concentration which inhibited 99% feeding (AFC_{99}) was found to be 18 ppm.

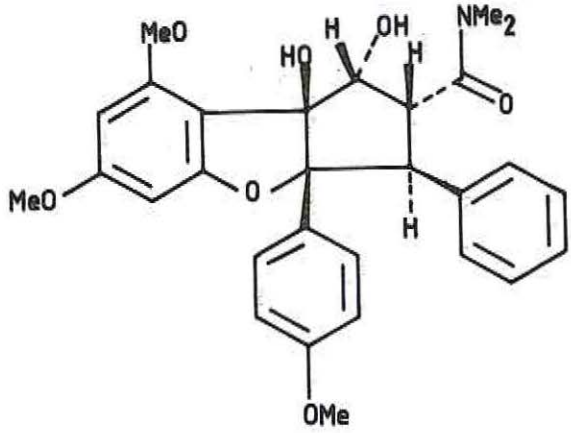

Figure 9

***Piper* sp.**

This plant species collected in Songkhla Province is a vine free from any insect infestation. It is now being cultivated for flowers for taxonomical classification.

The fruits and leaves of the plant were extracted separately with hexane, dichloromethane and then methanol. The most active fraction was found to be the hexane extract of the fruits. Test results indicated that the extract had a 'knock-down' effect and was effective by topical application.

Seven pure components had been isolated from the extract and are presently being examined spectroscopically for their structure at the time this report is being written. Two of these compounds, referred to as F 5.1.3.1 and F 6 (1.6) appeared to be active, and the combination of which exhibited the activity comparable to pyrethrin II (Table 2).

Table 2. The LD_{50} values for the compounds extracted from *Piper* sp.

Compound	LD_{50} (1 hr) µg/insect
F 5.1.3.1	1.6×10^{-2}
F 6 (1.6)	4.5×10^{-3}
F 5.1.3.1 + F 6 (1.6) (1:1)	1.83×10^{-3}
Pyrethrin II	1.97×10^{-3}
Crude hexane extract	1.04×10^{-2}

The results obtained warrant further investigation in the chemical structure and the field application of these active ingredients.

CONCLUSION

From this investigation, several chemical components which showed anti-feeding and insecticidal activities against *S. litura* larvae were isolated and identified from plants growing in Thailand. The most active anti-feedant was isolated from *A. odorata*, the crude extract of which gave satisfactory results in the field trials. The most active insecticidal compounds which have a 'knock down' effect were isolated from a *Piper* species. Two of these act synergistically to give the activity level comparable to pyrethrin II.

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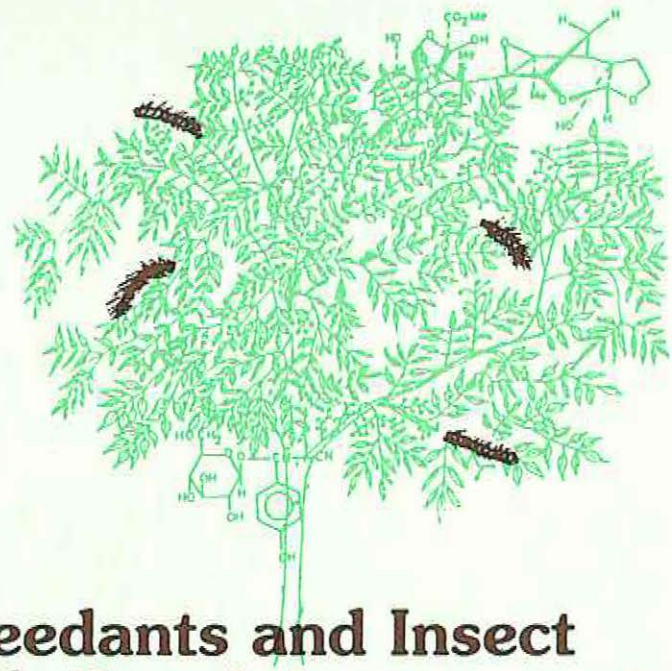

Anti-feedants and Insect Growth Regulators as a New Means of Control of *Schistocerca gregaria* in Egypt

4

Abdel Azim M. El-Gammal

Anti-feedants and Insect Growth Regulators as a New Means of Control Against *Schistocerca gregaria* in Egypt

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INTRODUCTION

Natural products are recently considered as the alternative hope to develop stable bioactive agents that are highly insect specific and environmentally acceptable than currently used pesticides.

The neem, *Azadirachta indica* was the first plant in which Pruthi (1937) discovered the repellent action against pests of stored-products. In 1962, Pradhan *et al.* demonstrated the anti-feedant action of neem kernel against *Schistocerca gregaria*. Afterwards, great attention has been focused on azadirachtin, the most proper limonoid derived from the neem tree *A. indica*. This was found to be a highly effective deterrent against a wide range of insect pests with the addition of hormone-like effects if ingested by insects (Rembold and Sieber, 1981).

The anti-feedant properties of other plant species against *S. gregaria* were reported by Rao (1982) in *Calitropis gigantia;* Singh and Pant (1980a) in *Crinum bulbispermum* and *C. asiaticum;* Singh and Pant (1980b) in *Hymenocallis littorallis;* Saxena (1980) in *Anethum sowa;* Bernays and Luca (1981) in *Stachytaropheta mutabilis;* Mohamed (1985) in *Lantana camara* and El-Gammal *et al.* (1988) in the wild plants, *Argemon mexicana, Solanum dobium, Zygophyllum simplex, Calotropis procera, Withania somnifera* and *A. indica.*

The main objective for locust control with IGRs is to prevent nymphal multiplication to suppress the outbreak of mobile swarms. To achieve this goal, precocene II can disrupt the natural balance of insect hormonal conditions by acting on juvenile hormone (JH) biosynthesis (Bowers, 1976). The role of precocene II on the early instars of *S. gregaria* was explored (El-Gammal, 1983; El-Gammal *et al.*, 1986).

JH-16, JH-17 and JH-18, administration to 4th nymphal instar of *S. gregaria* suppresses lipid but enhances protein and carbohydrate thus influencing energy production necessary for normal metamorphosis (El-Gammal, 1979; Taha, 1979). Also, the anti-moulting agents such as diflubenzuron and fenoxycarb induced morphogenetic and insecticidal effects in 4th and 5th nymphal instars of *S. gregaria* (Mariy *et al.*, 1981; El-Gammal and Taha, 1984; Taha and El-Gammal, 1985; El-Gammal *et al.*, 1988).

MATERIALS AND METHODS

Collection of the Wild Plants

During the joint ecological surveys between Egypt and the Sudan (1984–1985, sponsored by FAO) and the annual surveys in Eastern desert of Egypt around the Red Sea Coasts to Delta Tokar of the Sudan, the following plants were collected:

1. *Argemon mexican* L. (Papaveraceae).
2. *Solanum dobium* Fresen (Solanaceae).
3. *Zygophyllum simplex* L. (Zygophyllaceae).
4. *Calotrpis procera* Ait. (Asclepiadaceae).
5. *Withania somnifera* L. Dun. (Solanaceae).
6. *Azadirachta indica* (Meliaceae).

Anti-feedant Effects of the Wild Plants Against Locust

The leaves of the wild plants were rinsed in tap water, left to dry, ground, and 10 g of each were mixed with the organic solvents, petroleum ether, diethy-lether, chloroform, hexane, ethanol 70%, distilled water. The extracts were filtered and filtrates examined for their anti-feedant actions against the adult stage of *Schistocerca gregaria* as described by Butterworth and Morgan (1971).

Anti-feedant Effects Against Grasshoppers

Leaves powder of three wild plants were extracted by two methods, either by crude extract or by successive extracts in which 10 g of each powder were mixed in 100 ml of hexane for 48 hr and filtered. The remaining powder was mixed again in the second solvent, then successively with the others (El-Gammal and Ghoneim, 1989).

The successive extraction system produced results which were quite satisfactory. This extraction system with *A. mexicana* and *C. procera*

produced strong anti-feedant action against *Eyprepocnemis plorans plorans* (El-Gammal and Ghoneim, 1989).

Bioactivity of Some Fractions of Wild Plants

240 g of the fine powder of *A. mexicana* and *Z. simplex* were separately extracted with dichloromethane (CH_2Cl_2) for one day, filtered and the organic solvent evaporated.

A column of Florisil was slurry packed in hexane in a 2 cm dia. tube with stopcock and lass frit, and weighed crude extracts of the two plants were chromatographed by elution system of hexane and diethyl-ether (El-Gammal *et al.*, 1990a).

By this method six fractions (F) were obtained from each plant. The fractions were tested to disclose, their bioactivity against, *S. gregaria*, *Spodoptera littoralis*, aphid, and *Tribolium confusum* (El-Gammal *et al.*, 1990b).

Anti-feedant Effects of Fractions

The anti-feedant effects of the six fractions (F) were tested against the 5th nymphal instar of *S. gregaria*, 10 mg of each fraction was dissolved in 10 ml of each elution system by which the filter paper discs were impregnated then tested as described by Batterworth and Morgan (1971).

Toxic Effects of Fractions

The toxic effects of the fractions (F) were assayed by thin layer film technique against 1st and 2nd nymphal instars of *S. gregaria*, 4th larval instar of *S. littoralis*, adults of *Myzus percicae*, and adult of *Tribolium confusum*. Each thin film in a Petri dish consumed 9 mg of each fraction and the insects were kept on it for 24 hr. Thereafter, the thin films were kept for 18 days in room temperature followed by 24 and 48 hr outdoors to examine their residual effects (El-Gammal *et al.*, 1990).

Residual Effects of Plant Fractions

F_2, F_3 of *A. mexicana* and *Z. simplex* were kept under sunshine for 48 and 96 hr then the residual effects were measured against 1st and 2nd nymphal instars of *S. gregaria*.

RESULTS

The results showed that all the extracts of neem, *A. indica* were repellent to the adults. *A. mexicana* extracts in diethy-lether, chloroform, ethanol and distilled water produced 100% reduction in adult feeding. *Z. simplex* extracts in diethyl-ether, and distilled water resulted in 100% feeding reduction, while with pet. ether, hexane and ethanol, 70, 88.19, 85.19 and 82.35% feeding reduction were induced, respectively.

C. procera extracts in diethyl-ether and water produced 100% feeding reduction, the other extracts in chloroform, ethanol, pet. ether and hexane produced 85.19, 81.48, 74.07 and 55.56% feeding reduction, respectively.

S. dobium extracts in ethanol, distilled water, hexane, pet. ether, diethyl-ether and chloroform produced 98.04, 92.59, 88.89, 66.67, 33.33 and 22.22% reductions in adult feeding, respectively. In a respective manner, *W. somnifera* extracts in distilled water, diethyl-ether, chloroform, pet. ether, and ethanol resulted in 100, 81.48, 66.67, 59.26 and 18.5% feeding reductions (El-Gammal *et al.*, 1988).

The anti-feedant properties of both types of extracts against grasshoppers *Eyprepocnemis plorans plorans* were quite satisfactory (El-Gammal and Ghoneim, 1989). Successive extraction with the solvents, ethanol 70%, diethyl-ether, pet. ether, distilled water and hexane on the plant species, *A. mexicana* and *C. procera* produced higher percentages of feeding reduction against the adult stage compared to the crude extraction system.

The results showed that *A. mexicana* possesses anti-feedant properties. The percentages of feeding reduction were 61.4, 56.85, 59.1, 80.7 and 36.4% with fractions, F_2, F_3, F_4, F_5 and F_6 respectively. On the other hand *Z. simplex* acted as a stimulant since stimulus percentages with the same fractions were 120, 100.2, 116.9, 114.6 and 134.8%, respectively (El-Gammal *et al.*, 1990).

The thin layer film of fractions F_2 and F_3 of *A. mexicana* produced 100% mortality against 1st and 2nd nymphal instars of *S. gregaria*, 4th larval instar of *S. littoralis* and the aphids, *M. percicae*. F_4 induced 60 and 55% mortality against 1st and 2nd instar nymphs of *S. gregaria* and 90 and 100% mortality against 4th larval instar of *S. littoralis* and *M. percicae*, respectively. As for *T. confusum* F_2 produced 90% kill while F_3 induced only 50% mortality.

The thin film of fraction-2 (F_2) of *Z. simplex* induced 100, 90, 80, 95.5 and 30% mortality against 1st and 2nd nymphal instar of *S. gregaria*, 4th larval instar of *S. littoralis*, *M. percicae* and *T. confusum* respectively. F_3 produced 100% kill to all insects except for *T. confusum* where 70% mortality was obtained. F_4 resulted in 20, 70 and 62% mortality against 1st nymphal instar of *S. gregaria*, 4th instar of *S. littoralis*, and *M. percicae*, respectively. In conclusion, F_2, F_3 of *A. mexicana* and *Z. simplex* induced high toxicity to *S. gregaria*, *S. littoralis*, *M. percicae* and *T. confusum* (El-Gammal *et al.*, 1990).

After 48 hr the thin film of F_2 of *A. mexicana* caused 100% kill against both instars nevertheless this fraction did not show mortality after 96 hr. F_3 resulted in 100 and 40% mortality against 1st and 2nd nymphal instars and 200 and 60% mortality after 96 hr exposure to sunshine (El-Gammal *et al.*, 1990).

F_2 and F_3 of *Z. simplex* induced 100% mortality against 1st nymphal instar and 40% mortality against the 2nd after 48 hr exposure to sunshine, while these fractions resulted in 100% kill against the two instars after 96 hr exposure to sunshine (El-Gammal *et al.*, 1990).

OTHER RESEARCH ACTIVITIES ON NATURAL PRODUCTS AND INSECTS

Growth Regulators (IGRs)

The main objective in locusts control is to prevent nymphal multiplication in their breeding sites to suppress outbreaks of mobile swarms. Accurate and modern mechanisms to prevent gregarization and metamorphosis of nymphal instars are, therefore, of importance in locust control.

In the light of this, anti-juvenile hormone (JH) agents such as precocenes are extracted from the plant *Agaratum houstanianum* (Bowers, 1976). JH-analogues, urea and carbamate derivatives are relatively new groups that can also prohibit metamorphosis and embryonic development.

Administration of JH-17 and JH-18 to the early nymphal instars of *S. gregaria* suppresses lipid but enhances protein and carbohydrate thus influencing energy production necessary for normal metamorphosis. The effect with JH-16 was more pronounced (El-Gammal, 1979; Taha, 1979). Moreover, the metamorphosis of the last nymphal instar of *S. gregaria* was inhibited by JH-3183 (Taha and El-Gammal, 1990).

Precocene II when applied on early instars of *S. gregaria* and *Eyprepocnemis plorans plorans* produced various rates of abnormalities ranging from permanent nymphs to precocious adults. Precocene II also, causes adult sterility (El-Gammal, 1983; El-Gammal *et al.*, 1986).

Several other precocene II studies are underway. They are designed on the basis of previous findings that revealed the potential use of precocene II as a strong inhibitor to embyrogenesis, and its outstanding adulticidal effect.

Diflubenzuron causes insecticidal and morphogenetic effects in 4th instar nymphs (El-Gammal and Taha, 1984; Taha and El-Gammal, 1985; El-Gammal, 1990, in press).

The carbamate fenoxycarb affects last nymphal instar by increasing carbohydrate levels and suppressing lipid contents and cholesterol, thus lowering the level of ecdysone leading to failure of ecdysis. These results were indicated in *S. gregaria* and *S. littoralis* (El-Gammal *et al.*, 1988a, b).

Cycloheximid and gamma rays produced permanent nymphs (El-Gammal, 1983; El-Gammal *et al.*, 1988).

Future Plan for Natural Products

1. Search for more wild plants for testing during routine surveys of *S. gregaria*.

2. Studies on the bioactivity of the following plant groups as found in literature against locust:
   - — plants on which the complete life cycle can be accomplished.
   - — plants on which hoppers development is completed although considerable mortality is observed and adults do not mature sexually.
   - — plants on which hoppers can reach the last instar but mortality is considerable at the final moult or immediately after.
   - — plants on which only early instars survive.
   - — plants eaten only by the first instar hoppers.
   - — plants not eaten only by the first instar hoppers even when starved.

3. The final goal is to isolate and identify the most potent compounds (anti-feedant or toxic) in these plants and other indigenous plants prevailing in Egypt and the Sudan on which the desert locust cannot feed.

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Asarones from *Acorus calamus* L: Chemistry and Biological Activity Against Insects

5

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Asarones from *Acorus calamus* L: Chemistry and Biological Activity Against Insects

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INTRODUCTION

In recent years there has been increased interest in the discovery of new chemicals for insect control in order to replace synthetic chemicals which constitute environmental pollutants and to which many insects have developed resistance. New chemicals are, therefore, needed which are efficacious, safe, biodegradable, and economically viable.

One successful approach has been the study of natural products, particularly the allelochemicals, which play key roles in the chemical defense of plants. Various extracts of plant materials have been used for insect control from ancient times and nearly 2000 species of plants are known to be biologically active against insects (Crosby, 1966; Jacobson, 1975). About 250 compounds have been isolated from various natural sources (Koul, unpublished data) which have insecticidal activity; however, there are many others which act more subtly as insect anti-feedants and/or growth inhibitors. These natural products could be of potential value in insect pest control in several ways. They may be a source of chemical structures for screening. They may be sufficiently efficacious to be used directly or their efficacy may be improved by structural modification of the parent structure or finally the recognition of their mechanism-of-action may suggest new approaches to the development of chemicals for pest control (Plimmer, 1985). Therefore, screening of plant materials as insect control agents, followed by the isolation and modification of active principles becomes an important study and a contribution towards the goal of obtaining new insect control products.

One such extensive study has been that of the rhizomes of *Acorus calamus* L. (Araceae), the Indian sweetflag plant, which has attracted the attention of many workers owing to its insecticidal properties against a wide variety of insects (Dixit *et al.*, 1956; Pandey *et al.*, 1977; Teotia and Pandey, 1979; Sujatha *et al.*, 1988; El-Nahal *et al.*, 1989). The powder and extracted essential oil from the *A. calamus* rhizomes acts as

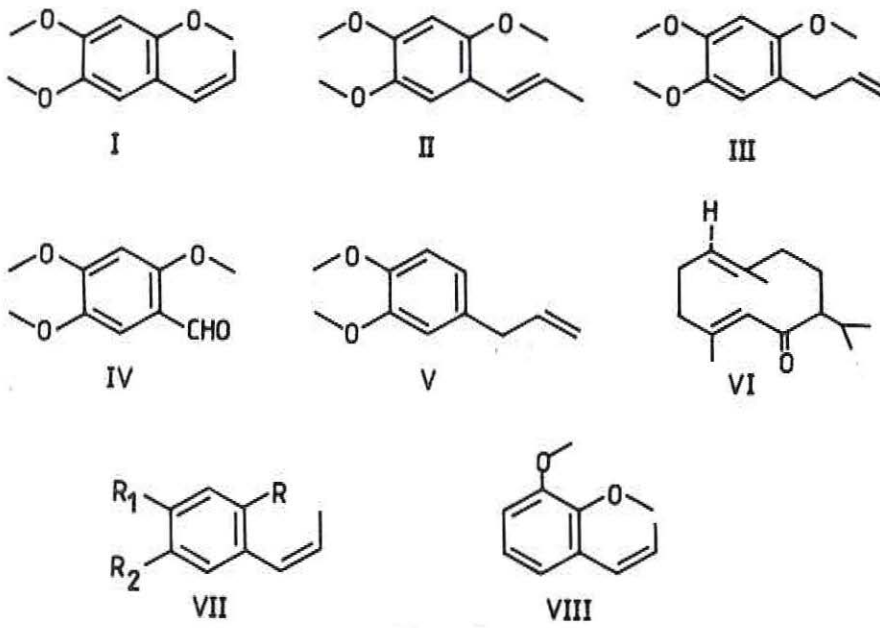

Figure 1

a contact or stomach poison, an anti-feedant, or a repellent to these insects (Saxena and Koul, 1978, 1982; Gillani *et al.*, 1988). More recently, sterilizing effects of the vapours of calamus oil have been reported in certain insects (Mathur and Saxena, 1975; Saxena *et al.*, 1977a,b; Koul *et al.*, 1977a,b; Koul, 1979; Schmidt and Borchers, 1981; Matolcsy *et al.*, 1981; Ramos-Ocampo and Hsia, 1986). The active principle responsible for sterilizing activity has been determined to be β-asarone (*cis*-1 propenyl- 2,4,5-trimethoxy benzene, Fig. 1, I) (Gildemeister and Hoffmann, 1966; Saxena *et al.*, 1971b; Schmitd, 1986). Recently, investigations on the anti-feedant and growth inhibitory effects of this plant material against lepidopterans have been carried out. This paper provides an overview of the studies on various effects of *A. calamus* or its isolates against insects.

CHEMICAL NATURE

A detailed chemical investigation using GC-MS analysis of the constituents of the rhizomes of calamus has been published (Mazza, 1985a,b), based on isolates from the oil and the alcoholic extract of dried rhizomes. More than 250 compounds have been identified including hydrocarbons, carbonyl compounds, alcohols, terpenes, and various oxidation products (Fujita *et al.*, 1971; Mazza, 1985a, b). A number of sesquiterpenes have also been isolated from the rhizome extracts (Niwa *et al.*, 1975). Patra and Mitra (1979) reported the isolation of 2,4,5-

trimethoxy benzaldehyde, 2,5-dimethoxy benzoquinone, and galangin along with the new cyclobutano lignan acoradin from similar extracts. Besides the known compounds, two new ones Z-3-(2,4,5-trimethoxy phenyl)-2-propanol and a phenyl indane have been isolated from the rhizome extracts (Saxena, 1986). As it is beyond the scope of this paper to describe all the compounds in detail, I will focus on those compounds which show biological activity against insects.

β-asarone (Fig.1, I, also referred to as *cis*-asarone) is a major constituent of India calamus (Guenther, 1952) though other isomers like *trans*-asarone (II), isoasarone (III), acaromone and asaraldehyde (IV) have been isolated from the oil (Patra and Mitra, 1981). β-asarone, which is a *cis*-I propenyl- 2,4,5-trimethoxybenzene, was isolated and structurally elucidated as early as 1937 (Sanjiva Rao and Subramaniam, 1937), although its synthesis dates to the late 19th century (Gatterman and Eggers, 1899). This compound remains the most active isolate from calamus oil, exhibiting anti-gonadal, anti-feedant, and growth inhibitory effects while *trans*-asarone induces anti-feedant activity alone. These two isomers constitute about 72% of Indian calamus oil by weight (Koul *et al.*, 1990b). However, the percentage of asarones varies, depending on the geographic source of the plant material. Isoasarone (III) isolated from *Piper futokadzura* has also been shown to inhibit feeding in *Spodoptera litura*, but has not been tested for bioactivity after isolation from *Acorus calamus* (Patra and Mitra, 1981).

Compounds such as methyl eugenol (V), acorogermacrone (VI) and asaraldehyde (IV) have been shown to attract male and female Mediterranean fruit flies and female oriental fruit flies (Jacobson *et al.*, 1976). All other studies regarding biological activity are primarily based on extracts or essential oil.

INSECTICIDAL ACTIVITY

Dried rhizomes of *A. calamus* are used for medicinal purposes, as an ametic, laxative and diuretic in Indian medicine (Chopra, 1956; Mazza, 1985), in the formulation of vermouths, liqueurs and bitters, and in veterinary medicine to treat anaemia and anorexia in cattle, yet its oil, extracts and isolates induce deleterious effects against a wide variety of insects. The potential of calamus oil or extracts as insecticides has been extensively studied for stored-grain pests like grain moth, *Sitotroga cerelella*, pulse beetle, *Callosobruchus chinensis*, granary weevil, *Sitophilus granarius*, rice weevil, *S. oryzae* , red flour beetle, *Tribolium castaneum* , and lesser grain borer, *Rhizopertha dominica* (Yadava, 1971; Teotia and Pandey, 1979; El-Nahal *et al.*, 1989). Chander and Ahmad (1986) applied

different doses of oil to seeds of green gram, *Vigna radiata*, for protection against *C. chinensis* and found 1 mg/kg offered a high degree of protection for 135 days. The active component (β-asarone), tested against another species *(C. maculatus)* killed all individuals when incorporated at 1% in diet (Janzen *et al.*, 1977). Toxicity due to asarones in *C. chinensis* , *T. castaneum*, *R. dominica* , *T. granarium* , and *Corcyra cephalonica* is also known (Agarwal *et al.*, 1973) but there is no substantial quantitative data recorded for the pure compounds. Paul *et al.* (1965) state that a petroleum ether extract of *A. calamus* rhizomes was inactive against *T. castaneum* adults using doses as high as 90 µg/ insect. According to recent studies it has been concluded that the period of exposure for stored grain pests is not as important as dosage (El-Nahal *et al.*, 1989).

Other insects reported to be susceptible to calamus oil include mustard saw flies, *Athalia proxima* (Pandey *et al.*, 1977), termites (Paul *et al.*, 1965), and kelp flies, *Coelopa frigida* (Ramos-Ocampo and Hsia, 1986b). Recently, petroleum ether extracts of calamus rhizomes were tested for mosquito larvicidal activity against *Culex quinquifasciatus*, *Aedes aegypti*, and *Anopheles stephensi*, yielding LC_{50} values of 33.7, 39.4 and 48.8 mg/l, respectively (Sujatha *et al.*, 1988).

However, other studies have shown that calamus oil or pure asarones are not insecticidal in a true sense as rapid poisoning does not occur after treatment (Koul, 1979), compared to most synthetic insecticides. In these cases delayed mortality may occur as a result of anti-gonadal, anti-feedant, or growth inhibitory effects.

OVICIDAL PROPERTIES

There are scant reports of the ovicidal activity of *A. calamus* against insects. Saxena and Srivastava (1972) were apparently the first to report the ovicidal effect of Indian calamus oil vapours on *Dysdercus koenigii*. At the highest dose of 1.5 µl calamus oil hatchability was reduced to 60% in 0–24hr-old treated eggs. Similarly to determine the ovicidal effects of a European variety of calamus oil and various propenyl benzenes, freshly laid eggs of *Oncopeltus fasciatus* were exposed to different levels of these substances (Ramos-Ocampo and Hsia, 1986a). β-asarone was not toxic (LC_{50}=1.93 mg/ml). Calamus oil as a whole was effective followed by *trans*-asarone (LC_{50}). It has been suggested that ovicidal action occurs only at the late 0.46 mg/ml (LC_{50}). It has been suggested that ovicidal action occurs only at the late stages of embryogenesis as most treated eggs contain fully developed embryos before they are defunct.

ANTI-GONADAL EFFECTS

Extensive studies of the anti-gonadal effects of *A. calamus* oil and its active principle β-asarone were conducted initially with whole essential oil and ultimately confirmed using isolate constituents. Preliminary observations of grain mixed with crushed fragments of calamus rhizomes or calamus oil in closed containers revealed significant reduction of progeny of stored grain pests (*T. castaneum, S. oryzae, C. chinensis, T. granarium,* and *Anthranus vorax*). Similarly administration of oil to freshly ecdysed and 5–6day old adults just prior to oviposition revealed no lethal effects but significantly reduced fecundity (Saxena *et al.*, 1976). Microscopic examination of the ovaries in treated insects showed significant regression in volume of the terminal oocytes in the vitellarium. Moreover, morphogenesis of the general ovarian pattern and follicular cells were also affected (Tikku *et al.*, 1978a,b). In extreme cases the entire vitellarium is lost and the germarium remains connected with the oviduct through a thin tube lacking cellular differentiation. These observations led to the conclusion that calamus oil was anti-gonadal and radically unlike JH analogues or other toxic chemosterilants.

Calamus oil also has bioactivity against male insects, causing agglutination and immobilization of sperm in mature testes (Koul *et al.*, 1977b). Sperm thus affected exhibit extreme elongation and nuclear deformity. The number of sperm which pass into the female genital tract was meagre and their passage probably resulted only due to mechanical forces *in copula*. Spermatogenesis studies in *Dysdercus koenigii* (Saxena *et al.*, 1977a) under the influence of calamus oil treatment (5 to 20 µl/1500 cm^3 area in vapour form) revealed deleterious effects. Various morphological effects are evident at 15 µl/ 1500 cm^3 (Fig. 2). Failure of secretions in interstitial and spongy cells detected through the Sudan Black B technique was a characteristic feature. An incidental study during this period, however, revealed no abnormal effects on the chromosomal pattern of testes cells (Koul *et al.*, 1977c).

Isolation of the active compound β-asarone via routine physico-chemical methods and subsequent bioassays has yielded the same results as that of the whole oil (Saxena *et al.*, 1977b). In order to locate the exact step in the sequence of reproductive impairment following β-asarone treatment, neurosecretory cells (NSC) were studied through Victoria blue and Aldehyde fuchsin staining, and granules were scored quantitatively in both normal and treated insects. No decrease in cellular secretion was recorded except at very high doses of calamus oil

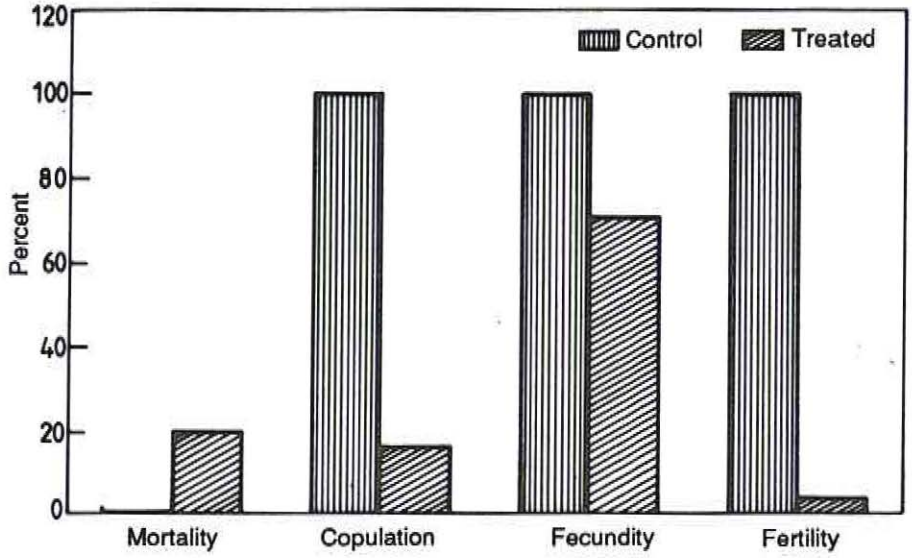

Figure 2

treatment (>30 µl/1500 cm^3 area in vapour form), and it was concluded that the candidate compound did not interfere with the release of moulting hormone. However, at higher treatment levels insects were lethally entangled in their half shed exuvia which may have resulted from a slight inhibition in release of NSC materials, in turn delaying the release of moulting hormone and ultimately impeding ecdysis (Koul, 1979).

Similarly, there was no anti-JH effect because precocious adults were not obtained and cellular necrosis of the corpora allata *in vitro* (similar to that from precocene treatment) was not observed (Muller *et al.*, 1979). Physiological reversal of the effect via topically-applied farnesyl methyl ether, a JH analogue, was unsuccessful (Saxena *et al.*, 1977b). Thus, the major mechanism of action was the inhibition of secretions from interstitial cells which in turn impaired spermatogenesis and vitellogenesis and/or caused resorption of oocytes.

A hypothesis proposed at this point was that JH stimulates interstitial cell secretion, probably of steroidal nature, which in turn induced follicular cell stimulation and ultimately yolk deposition in the oocytes. If β-asarone blocks the stimulus between interstitial cells and oocytes (Fig. 3), there would be a severe effect on the ovarian maturation and ultimately on fecundity (Koul, 1979; Saxena and Koul, 1982). To answer the question of which of the substituent groups in the

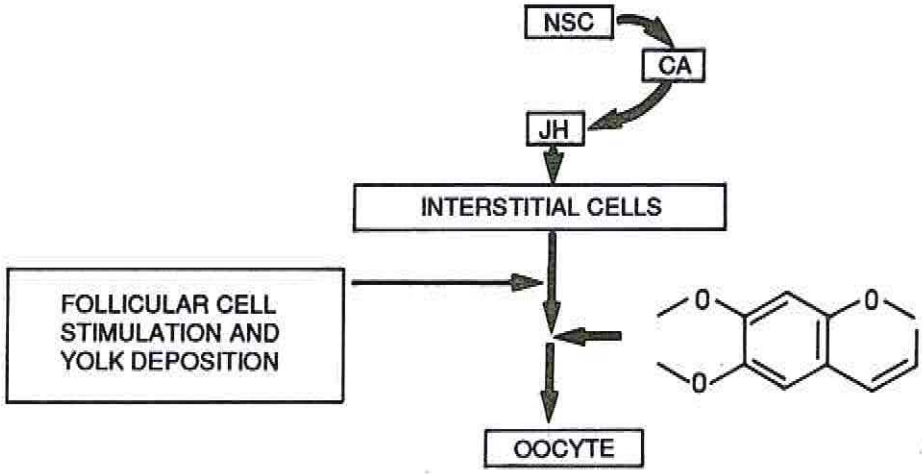

Figure 3

aromatic ring or side chain of β-asarone is responsible for anti-gonadal activity, various derivatives were prepared. Our structure-activity studies showed that methoxylation at position R in the ring was essential for activity (Fig. 1, VII), which was further enhanced by methoxylation at R_2 (Saxena *et al.*, 1977b; Koul, 1979). Comparison of the derivatives also revealed that the *cis* configuration of the double bond in the side chain was also a requirement for activity. Further studies with asarones and their analogues (Matolcsy *et al.*, 1981) against *Dysdercus cingulatus* also suggest suppression of fecundity due to β-asarone and related compounds methxylated at position R. Similar observations have been made for another derivative, 2,3-dimethoxy propenylbenzene (Fig. 1, VIII) (Ramos-Ocampo and Hsia, 1986b). In these studies, β-asarone was also the most active substance inducing sterility in *Oncopeltus fasciatus*, followed by 2,3-dimethoxy propenylbenzene. Both compounds significantly reduced fecundity and hatchability, disrupted maturation and depressed other reproductive activities. They support the earlier conclusion that *trans*-asarone is not an anti-gonadal component of calamus oil. Hymenopterans have also been shown to be susceptible to sterilization by calamus oil treatments, particularly in ants where a detailed investigation has been conducted (Schmidt and Borchers, 1981).

ATTRACTANT PROPERTIES

Jacobson *et al.* (1976) found that a distillate of essential oil of *A. calamus* rhizomes was highly attractive to female Mediterranean fruit flies,

Ceratitis capitata, female melon flies, *Dacus cucurbitae*, and male and female oriental fruit flies *D. dorsalis*. The active components isolated from the distillate were identified as β-asarone, acoragermacrone (VI), and asaraldehyde (IV). Surprisingly, β-asarone was very attractive to male oriental fruit flies instead of being toxic. Of the three positional isomers of asaraldehyde, only the 3,4,5-isomer showed activity. The concentration used for these compounds was 0.1% in water. Similarly, *trans*-asarone isolated from the surface wax of carrot, *Daucus carota*, leaves stimulates oviposition in the carrot rust fly, *Psila rosae* (Stadler and Buser, 1984).

ANTI-FEEDANT AND GROWTH INHIBITORY EFFECTS

Little is known about the effect of calamus oil/extracts/isolates against lepidopterans. Recently, it was demonstrated that calamus oil deterred feeding and growth of *Spodoptera litura* larvae when sprayed on castor foliage (Koul, 1987). A dose-response relationship was established using food acceptance, feeding ratio, weight gain, and larval development as parameters in choice and no-choice tests. Calamus oil at 0.5 and 1.0% concentrations significantly deterred feeding and inhibited growth in early third instar larvae (Table 1). In order to corroborate this data, to isolate the active components responsible for these activities and to determine the mode of action, a detailed investigation was carried out using the variegated cutworm, *Peridroma saucia*. The other study

Table 1. Incidence of anti-feedant and growth inhibitory effects of *Acorus calamus* L. oil against *Spodoptera litura* larvae

Choice Test

Treatment (%)	Acceptance of foliage after 24 hr (%)		Weight gain after 5 days (mg)
	Treated	Untreated	
0.0	–	8.2	743.5
0.25	0.3	6.3	510.4
0.50	0.0	9.3	405.4
1.00	0.0	1.7	85.2

No-Choice Test

Treatment (%)	Feeding ratio after 24 hr (mg)	Weight gain after 5 days (mg)
0.25	0.20	294.0
0.50	0.21	184.1
1.00	0.0	10.0

Feeding ratio calculated as test/control consumption.

regarding growth inhibitory effects is that of Khan and Borle (1989) on pulse beetle attacking stored gram, *Cicer arietnum* where calamus rhizome powder arrested developmental activities in these insects.

Initial studies with calamus oil against first to fifth instar *P. saucia* larvae showed significant decreases in food consumption and larval growth in a dose dependent manner, whether incorporated in artificial diets or applied to cabbage leaf discs (Koul and Isman, 1990a). Nutritional analysis experiments with 3rd instar larvae revealed both anti-feedant and growth inhibitory effect at 20 μg/insect dose by topical application and 1250 ppm in diet. In feeding experiments, severe reductions were observed in RCR (Relative Consumption Rate) and RGR (Relative Growth Rate), with lesser corresponding differences in gross and net dietary utilization and approximate digestibility. In topical studies, only dietary utilization, but no consumption, were significantly affected (Koul and Isman, 1990a). The toxic and anti-feedant actions were apparently somewhat independent of each other.

Two active components isolated from the oil via fractional distillation and subsequent reverse phase high pressure liquid chromatography (HPLC) were identified as *cis*- and *trans*-asarone (Fig. 1, I, II), which were evaluated in detail against *P. saucia* larvae (Koul *et al.*, 1990b). It was confirmed that the two isomers have substantial differences in bioactivity. While *cis*-asarone is toxic in addition to having anti-feedant action, *trans*-asarone has anti-feedant activity alone with no appreciable toxicity (Koul *et al.*, 1990b). At the 750 ppm dietary level, *cis*-asarone reduced the growth of 4th instar larvae with a 45% decrease in net dietary utilization. Significant reductions in RGR and ECI were observed (Fig.4), with a 40% decrease in ECD following topical application of 10 μg/insect dose. In a leaf disc choice test IC_{50} values for *cis*-asarone were 2.5 $\mu g/cm^2$ for 4th instar larvae and 4.0 $\mu g/cm^2$ for 5th instar larvae. On the contrary, *trans*-asarone did not show any effects on dietary utilization in nutritional experiments, but anti-feedant effects were observed at higher concentrations in leaf disc tests. IC_{50} values of 17.5 $\mu g/cm^2$ and 22.0 $\mu g/cm^2$ for 4th and 5th instar larvae respectively were recorded (Koul *et al.*, 1990b).

In investigating the mode of action of β-asarone as an insect growth inhibitor, it has been established that the bioactivity of this allelochemical is synergized by a mixed-function oxidase (MFO) inducer, the monoterpene menthol, and antagonized by the well known MFO-inhibitor piperonyl butoxide. Formation of a bioactive epoxide was confirmed by the identification of asarone epoxide and asarone diol in frass (Fig. 5) of *P. saucia* larvae. This was confirmed further by

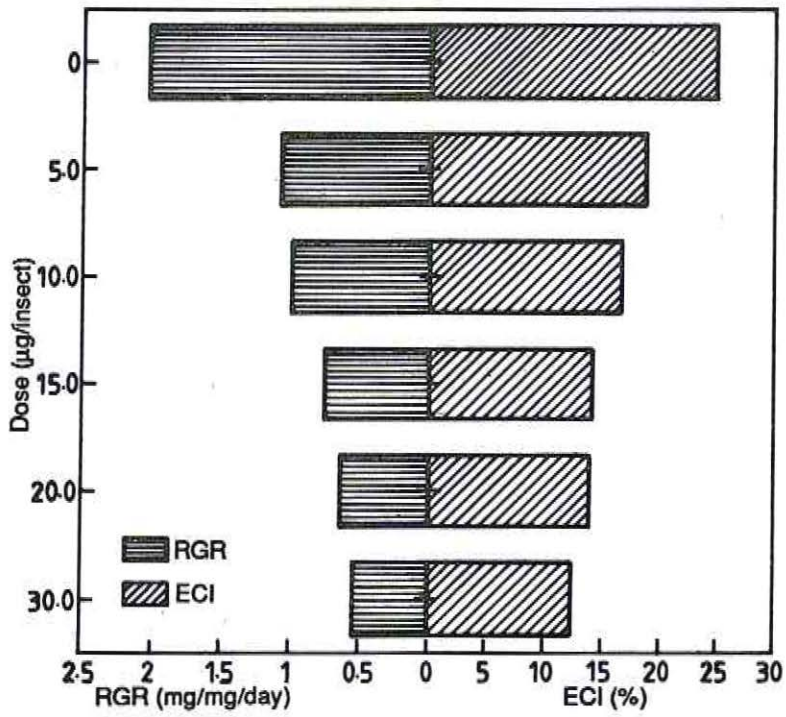

Figure 4

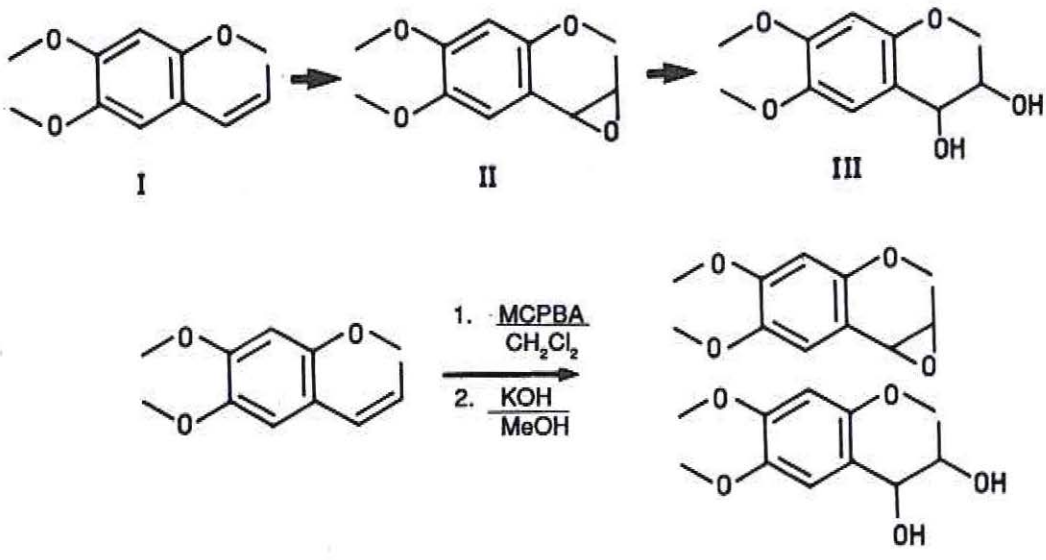

Figure 5

synthesizing asarone epoxide and asarone diol in the laboratory (Fig. 5). GC-MS analysis was used to correlate the fragmentation pattern of synthetic and frass extracted metabolites (Koul *et al.*, 1990c). This apparently is the first demonstration of synergism between two natural products (β-asarone + menthol) where mechanism involves the induction of enzymes usually involved in detoxication (Koul *et al.*, 1990c).

In summary, *A.calamus* is an effective anti-gonadal, anti-feedant and growth inhibitory plant material, where the bioactivity is due to the presence of two active principles, *cis*- and *trans*-asarone. While *cis*-asarone (β-asarone) is a toxic substance inducing nonspecific toxicity through oxidative activation by mixed-function oxidases, *trans*-asarone acts as an anti-feedant allelochemical only. It is apparent from the wide range of effects seen that *cis*-asarone has a general toxic action, unlike the precocenes (the anti-JH compounds) which also act through oxidative bioactivation but specifically affect the corpora allata (Pratt *et al.*, 1980). A generalistic effect of asarone is probably due to the stable epoxide which is apparently able to accumulate to some extent in different tissues albeit dependent on stage and time of application.

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Plant Extracts with Anti-feedant Activity Against *Nudauleria belina*

6

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Plant Extracts with Anti-Feedant Activity Against *Nudauleria belina*

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INTRODUCTION

A wide range of lepidopterous insects are pests of economic importance in Tanzania. The destruction caused by feeding on leaves and stems of both cash and food crops such as coffee, tobacco, cashewnut, cotton, cabbage, citrus fruits, potatoes and mangoes is quite extensive. Insect pest control, thus, deserves high priority in Tanzania. At present, it depends largely on the use of insecticides. However, because of the problems of insect resistance, safety and environmental pollution associated with insecticides, there is an urgent need to develop alternative methods of controlling insect pests. Therefore, the present study reports on our preliminary search for anti-feedants against the larvae of *Nudauleria belina* which defoliate mango and cashewnut crops. These investigations have not been reported in literature.

MATERIALS AND METHODS

Plants locally known to have medicinal and/or repelling properties against insects were collected from various parts of Tanzania and identified in the Botany Department of the University of Dar es Salaam.

Leaves, stem bark, seeds and root bark were air dried and crushed. The crushed material was cold extracted in turn with petrol (40–60°C) or hexane, dichloromethane or chloroform and finally methanol. The respective extracts were concentrated under reduced pressure for subsequent bioassay and chemical analysis.

Caterpillars in their third instar were collected from their host plants, namely, *Mangifera indica* and *Anarcadium occidentale*. Some of them were reared in a cage until adult moth emerged. These were identified by the Department of Entomology, National Museums of Kenya as *Nudauleria belina* (Westwood) (Saturnidae) also known as anomalous emperor moth.

Anti-feeedant test was carried out using the leaf disc method (Yano, 1986). Thus, fresh mango leaves of 2.5 cm dia. were dipped into acetone or hexane solution of the extracts. The control disc was dipped in the pure solvent. The discs were left to dry under a drift of air. The treated disc (TD) and the control disc (CD) were then introduced into a cage (10 x 10 x 10 cm) made of wood and wire mesh. The larvae were placed in between the TD and CD. The experiment was carried out at ambient temperature. After 3 hr, the discs were removed and the eaten areas measured. Each test was done in triplicate. The relative anti-feedant percentage (RAP) was calculated as follows:

$$RAP = \frac{\text{(-Average \% of consumed TD)}}{\text{(Average \% of consumed TD + Average \% of consumed CD)}} \times 100\%$$

where, TD = Treated disc and CD = Control disc.

The RAP values were then coded as follows: 0–49 (–) no activity; 50–59 (+) poor activity; 60–79 (++) moderate activity; 80–89 (+++) good activity and 90–100 (++++) very good activity.

Phytochemical screening was carried out using standard methods as follows:

In the petrol extracts we tested for the following classes of compounds:

Sterols and triterpenes (Harborne, 1976), basic alkaloids (Smolenski *et al.*, 1972), flavonic aglycones (Geissman *et al.*, 1955), anthracenoside aglycones (emodin and emodols) (Peyer, 1931) and coumarins (Gasparis and Manela, 1944).

In the methanol extract we tested for: polyphenols (tannins) (Farnsworth, 1966), alkaloid salts (Smolenski *et al.*, 1972), polyphenolic glycoside (anthracenoside) (Gasperis and Manela, 1944) and sterol glycosides (cardiotonic and saponins) (Combie *et al.*, 1944).

Where a positive reaction was observed the relative rating +, ++, +++, and ++++ was used in accordance with the relative intensities.

RESULTS AND DISCUSSION

The results of the anti-feeding activities of various plant extracts are shown in Table 1. It is observed that various plants belonging to

Table 1. Anti-feedant activity of extracts from some Tanzanian plants[1]

Plant species	Family	Part of the plant	Activity PE[2]	CH_2Cl_2	H_3OH
Annona reticulata	Annonaceae	stem bark	+	+	+
Annona muricata	Annonaceae	stem bark	++	++	++++
Andansonia digitata	Bombaceae	seeds	+	+	+
Cassia didymobotrya	Caesalpinaceae	leaves	++	+++	–
Albizia anthelmintia	Caesalpinaceae	stem bark	++++	–	++
Cassia occidentalis	Caesalpinaceae	leaves	++++	++	+
Dichrostachye cinerea	Caesalpinaceae	stem bark	++	++	–
Kigelia africana	Bignoniaceae	stem bark	+++	–	++
Balanites aegyptica	Balanitacea	stem bark	++++	++	+
Cussonia arborea	Araliaceae	stem bark	+	–	–
Sesbania bispinosa	Fabaceae	whole plant	++	+	++
Vismia orientalis	Guttiferae	stem bark	++	+++	++
Cucumis sativus	Cucurbitaceae	seeds	++++	++++	+++
Mirabilis jalapa	Nyctaginaceae	plants	–	–	++
Boerhavia diffusa	Nyctaginaceae	roots	–	+	+
Eucalyptus sp.	Myrtaceae	stem bark	–	++++	++++
Azadirachta indica	Meliaceae	stem bark	+++	+++	++++
Plantago major	Plantaginaceae	whole plant	++	+++	+
Gardenia jovis tonantis	Rubiaceae	stem bark	–	–	++
Cinchona sp.	Rubiaceae	stem bark	+	+	++
Vengueria infausta	Rubiaceae	stem bark	–	++	+
Toddalia asiatica	Rutaceae	root bark	++++	+++	+
Rauwolfia mombassiana	Verbanaceae	stem bark	+	++	+
Celtis durandii	Ulmaceae	stem bark	++++	++++	+++
Cissus quadrangularis	Vitaceae	plant	++++	+++	++
Parinari exelsa	Chrysobalanceae	stem bark	–	++	+

[1]A 1% solution of crude extracts in acetone or hexane for polar and non polar fraction respectively was used for the anti-feedant test.
[2]PE, Petroleum ether 40–60°C.

Table 2. Anti-feedant activity of extracts from Tanzanian plants

Plant species	Part of plant	Extracting solvent	Activity Conc. 1%	0.5%	0.25%
T. asiatica	root bark	PE[1]	++++	++	++
C. occidentalis	leaves	PE	++++	++++	++
A. anthelmintica	stem bark	PE	++++	++++	++
A. muricata	root bark	CH_3OH	++++	++++	++
C. durandii	root bark	PE	++++	++	+
C. quadrangulari	plant	PE	++++	++	+
C. sativus	seeds	PE	++++	ND[2]	ND
Eucalyptus spp.	stem bark	CH_3OH	++++	ND	ND

[1]PE, petroleum ether (40–60°).
[2]ND, not determined.

Table 3. Phytochemical screening of active extracts

Plant species	Extraction solvent	Part used	Activity (RAP %)	Class of compounds screened: Emodins and Emodols	Flavone aglycones	Coumarins	Sterols and triterpenes	Basic alkaloids	Saponins	Tannins	Alkaloid salts	Steroids glycosides (Cardiotonics)	Anthrocyanocides
A. muricata	CH_3OH	Stem bark	100	++	++	+++	+++	–	–	+++	+++	++	–
C. occidentalis	PE	leaves	100	+++	+++	–	+++	–	–	–	–	–	–
Eucalyptus sp.	CH_3OH	stem bark	90	+	–	–	–	–	+++	+++	–	+++	+++
T. asiatica	PE	root bark	100	–	++	+++	+	+++	–	–	++	–	–
T. asiatica	CH_3OH	stem bark	50	–	++	+++	+	–	–	–	++	–	–
T. asiatica	PE	stem bark	95	–	+	++	+++	+	++	–	–	–	–
C. durandii	PE	stem bark	100	–	+	++	+++	++	–	–	–	–	–
C. durandii	CH_3OH	stem bark	90	–	++	+++	++	–	–	–	–	–	–

different families deter feeding of the *Nudauleria belina* larvae. However, it is not possible to correlate the level of activity with the family, plant or the part of plant used. This observation is not unexpected since even the most widely studied pest, African armyworm *Spodoptera exempta* anti-feedants have been isolated from at least seven plant species belonging to six families (Asakwa *et al.*, 1980; Kubo *et al.*, 1980a; Kubo *et al.*, 1980b, Supplement 1985; Hassanali *et al.*, 1984; Kubo *et al.*, 1977; Simmonds *et al.*, 1985). Also it is not possible to correlate the activity with any extraction solvent.

Plant extracts which were active at a concentration of 1% (w/v) were further tested at 0.5 and 0.25%, respectively. Most of the extracts registered poor activity at 0.25%. Results are shown in Table 2. Extracts which exhibited high activity (3+ and 4+) were subjected to phytochemical screening. Results are summarized in Table 3. It is evident that several classes of compound deter feeding of *Nudauleria belina* larvae. This observation may suggest the pest to be monophagous; our experience has shown that the pest feeds on Anarcadiaceae family.

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The Commercialization of Neem

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The Commercialization of Neem

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INTRODUCTION

The need for compounds which have a combination of excellent insecticidal activity and desirable low toxicity to plants and animals is universal. Compounds are needed with greater insecticidal activity, better selectivity, lower environmental impact, low production costs, and effective against those insects which resist other insecticides. Neem may be a source of several such compounds.

The neem or nim tree (*Azadirachta indica* A. Juss) is native to the Burma/India region, and is known by a number of other common names including Margosa and Indian lilac. It has been spread throughout the tropics and subtropics by Indian migrants, and has become the tree of choice for reforestation for firewood, charcoal, and construction poles, due to support by USAID, GTZ, CARE, and other organizations.

Neem is adapted to altitudes between 50 and 100 m, tolerates poor, dry soils, but is susceptible to frost and water-logged soils. Millions are now growing along roadsides, and scattered across countrysides in most tropical and subtropical areas. It does not compete well with certain other species, and appears to do poorly in plantations. Whether this is due to limiting factors such as light, water, or nutrients, or to allelopathic effects is not known. It is easily propagated using fresh seed, but seeds remain viable for only two weeks to a month, depending on storage conditions.

Trees begin to bloom at different ages. In India, they normally become productive in 5 to 7 years. In Haiti, trees begin to bloom in 3 years. Flowers are small, lilac coloured, and develop into yellow-green fruit the size of olives. In India, flowering occurs from January through April, with fruit maturing from May to August. In some other countries, flowering and fruit set occur twice a year.

COMMERCIALIZATION

Various parts of the neem tree have been used for centuries. Commercialization of neem products dates back 4000 years to the Vedic period of India. Sanskritic writing from this period refer to its use by wandering medical practitioners, in primitive treatments for a wide variety of disorders.Seeds and leaves are still used in homes and on farms to protect stored food, feed and seed. Crude aqueous and ethanolic extracts have been used to control a wide spectrum of insects. Commercial neem products include toothpaste, soap, shampoo, and other skin and animal care products.

All parts of the tree have biological activity. Over 100 compounds have been isolated and their structures elucidated. The majority of these are triterpenes of the limonoid type. Azadirachtin is the most biologically active, and found in the greatest concentrations. It is a very large, complex molecule, with a molecular weight of 720. Due to its complexity, azadirachtin is not easily synthesized, and synthesis is not likely to be cost-effective. The best source of azadirachtin will likely always be the seed of the neem tree.

One of the first efforts to commercialize azadirachtin in the USA was by Vikwood Botanicals, Inc. of Sheboygan, Wisconsin, which has since been purchased by W. R. Grace. An azadirachtin containing insecticide was marketed under the trade name Margosan-O. This was an ethanolic extract from neem seed, with 3000 ppm azadirachtin and approximately 20% lipid-like solids, to act as emulsifiers and sunscreen. It was applied as a foliar spray or soil irrigant with systemic action, for non-food crops. They have announced another product under the trade name Margosan, with less oil and quicker and more effective systemic action. A third product has been announced, called Margosan-D, which is described as a dust of wood flour, neem cake and urea, to be used as a soil conditioner and fertilizer.

The agrichemical company Rohm and Haas filed for a European patent in 1986 on hydrogenated forms of azadirachtin, which have enhanced ultra violet stability. NPI is a biotechnology firm based in Salt Lake City, Utah, currently performing the toxicity testing required for registration of an azadirachtin-based insecticide. Safer Ltd. of Canada is investigating neem extracts as a possible addition to their line of biological products.

EXTRACTION AND PURIFICATION

Numerous ways have been used over the years to extract compounds from the neem seed. Each method gives different yields and different components. Here is an example of a relatively efficient extraction method.

- Grind air dry, whole seed to .1 to 10 mm diameter.
- Extract four times with a non-polar solvent (aliphatic hydrocarbons such as petroleum ether or hexane are preferred) to remove seed oils and fatty acids.
- Extract the meal with polar solvents (aliphatic alcohols such as ethanol and methanol are preferred). Azadirachtin and other active compounds are removed in this fraction.
- Remove sugars, and insoluble proteins from the extract using ethyl acetate.
- Remove the solvents by roto-evaporation.

This procedure yields an extract with 15 to 50 % azadirachtin. The rest is thought to be a mixture of similar type triterpenoids, many of which have insecticidal activity, and inert residual plant material.

Crude aqueous or ethanolic extracts are easy to do and could be made and used 'as is'. Further purification, if desired, can be achieved with flash, open column, droplet counter current and high performance liquid chromatography.

There are considerable yield data in the literature, but comparisons are difficult because of a lack of consistency in methods and in reporting. For example, it is often reported that the average neem tree yields 25 to 50 kg of seed. It is not specified whether that is fresh, air dry or oven dry seed. Further, the azadirachtin content of seed from various countries has been reported, but processing methods and storage conditions can affect the azadirachtin content, as can the extraction method.

An analysis of whole, air dry seed from a number of countries, including India, Togo, Haiti, Puerto Rico, and Niger showed the azadirachtin content averaged 0.3% on a dry weight basis, using hexane to remove oils, followed by four washes with ethanol to extract azadirachtin. Reports of 0.6% azadirachtin are not uncommon, but it is assumed this is on hulled or decorticated seed. Since the hulls make up just under 50% of the dry seed weight, azadirachtin content would be approximately 0.6%.

AFLATOXIN PROBLEMS

The most economical way to gather neem seed is to pick it up off the ground at the end of the season, after most of the fruit flesh is gone, leaving relatively clean seed. However, such seed has been found to often carry the fungus *Aspergillus favus* and *Aspergillus parasiticus*. These fungi cover the kernel inside the seed shell with yellow-green mycelium, and decay away the seed. More importantly, the mould produces some very potent mycotoxins, especially aflatoxin. Aflatoxin is a strong carcinogen, and also causes aflatoxosis if ingested by animals. When contaminated neem seeds are extracted, the aflatoxin is removed with the azadirachtin. The more the azadirachtin is purified and concentrated, the more concentrated the aflatoxin becomes. Separation of aflatoxin from azadirachtin is possible, but too costly. Several procedures effectively destroy aflatoxin, but they also destroy azadirachtin.

When and how the initial fungal infection of neem seeds takes place is not known, but it probably happens after the seed reaches the ground. Fresh fruit on the tree are usually not infected. Contamination appears to occur between when the fruit falls from the tree and when it is dried and stored.

Once the seed has been harvested, it must be stored under conditions least favorable to mould growth. The single most important factor contributing to mould growth is moisture content. Seed should either be extracted immediately, or stored under cool, dry conditions. It has been found that the fungus will not invade the seed when moisture content is in equilibrium with a relative humidity of 70% or less. Seed should be dried to below 15% moisture within 24 hours of harvest. There are reports that neem seed should not be sun dried, due to photodegradation of azadirachtin. It is also reported that high drying temperatures also destroy azadirachtin.

The causal organisms can be identified by examination under a microscope. Also, aflatoxin fluoresces greenish-yellow under U.V. light. This is proof positive, but not proof negative, since small but significant amounts are undetectable under U.V.

EPA has no set tolerance limits for aflatoxin in insecticides. (For comparison, FDA allows 20 ppb aflatoxin in peanut butter, but only 2 ppb in milk.) Potentially, aflatoxin poses a much greater threat than does azadirachtin. Of the tests required by EPA to register an insecticide

for use in the U.S.A., the one most sensitive to aflatoxin is the Ames mutagenicity test, which looks for mutations in five strains of bacteria.

OTHER PROPERTIES

Photostability

Azadirachtin forms used to treat field crops are subject to photodegradation by sunlight and ultraviolet radiation. Acetone solutions of this compound exposed for seven days showed more than 50% reduction in activity. After 16 days, it had zero activity. Hydrogenation increases the photostability, with no loss in insecticidal activity.

Neem Supply

Thanks to reforestation efforts supported by USAID and other agencies over the past several decades, there are millions of neem trees in production. Most are found along roadways, in windbreaks, and scattered across the country side. At present, there would appear to be no reason to establish additional trees or turn to plantation production, unless superior genetic material can be identified.

Insect Resistance

There are concerns that insects will eventually develop resistance to neem extracts, just as they have to nearly all other insecticides. There are few hard data, but activity of esterase and multifunction oxidase enzyme in diamond back moths did not change after 35 generations of exposure to neem seed extracts. No sign of resistance was observed in feeding and fecundity trials.

Beneficial Organisms

Not a great deal of information is available in this area, but a few reports have appeared in the literature. In a study at the University of California at Riverside, neem extract was sprayed directly on adult bees, using field dosages up to 4478 ppm a.i. per hectare. It was found to not harm bees at concentrations well above the dosage of 20 ppm recommended for control of the gypsy moth. In another study comparing the effects of neem extracts on spider mite *Tetranychus cinnabarinus* and on its predator mite, *Phytoseiulus persimilis*, no mortality to predator females resulted from up to 0.5% ethanol, 0.3%

acetone, and 0.05 % methanol extracts, formulated in water solutions, but even the lowest concentrations of pentane extracts caused mortality.

Neem products may actually benefit earthworms. Greenhouse studies where neem leaves and seeds were ground and incorporated into potted soil containing earthworms, showed a 25% increase in young earthworms over the untreated pots. In similar studies in the field, earthworms did not increase in number, but they did in average body weight.

EFFICACY TESTING OF NEEM EXTRACTS

NPI has recently begun a series of trials to evaluate the efficacy of various neem extracts as potential insecticides. The following is a summary of the efforts and findings todate.

Experimental Systems Used:

- Hemocoel injection studies
- Two-way choice leaf disc bioassay
- Antifeedant diet assay
- Whole plant, clip cage experiments
- Greenhouse cage experiments
- Field experiments

Experimental Preparations Evaluated:

- Production neem extract (PE)
- Hydrogenated PE
- Purified azadirachtin (95%)
- Hydrogenated azadirachtin

Experimental Pests:

- Fall armyworm (*Spodoptera frugiperda*)
- Tobacco budworm (*Heliothis virescens*)
- Colorado potato beetle (*Leptinotarsus decemlineata*)
- Sweetpotato whitefly (*Bemisia tabaci*)

Tentative conclusions:

1. The antifeedant activity of neem extract is an interesting property, but of limited commercial significance.
   - There is considerable variation between pests in antifeedant activity.

- Generally, high concentrations are required to inhibit feeding.
- This activity appears to be short lived at economically competitive concentrations.

2. Larvicidal activity may be the most important commercial characteristic of neem extracts.
   - Many important pests appear to be affected at commercially competitive concentrations.
   - Efficacy is reproducible.
   - Typically, there is no quick kill. Effects are seen after 3 to 15 days, depending on the pest and timing of application.

3. Adulticidal activity of neem extracts are only at high rates of application, and they tend to be variable. Lack of quick knockdown poses a challenge for commercial markets where rapid adult kill is important or expected. Pyrethrum could be added to provide quick knockdown.

4. The systemic activity of neem extracts deserves detailed study. It could be an extremely important component of efficacy in commercial use if it is 'quantitative' and widespread across plant taxa.

FACTORS LIMITING LARGE SCALE COMMERCIALIZATION

Handling

Mostly hand labor is used. Fruit is picked by hand, depulped by hand, cleaned by hand, decorticated by hand.

By products

Support industries are needed to use the by products, including tons of oil which is too bitter and smelly for cooking or even burning. There are reports of a new process to produce colourless, odourless, and debittered neem oil, suitable for cooking, and heating. Hopefully this process will be cost-effective.

Potential by-products are too numerous to mention. India already has a rather large neem soap industry. Both India and West Germany make toothpaste and powder from neem extracts. India makes animal care products, such as shampoos. Oils are also used for skin-care products, and could be used to produce lubricants and waxes. Potential

pharmaceuticals include antimalarials, febrifuges, antihelmintics, antiseptics, and even spermatocides. Neem extracts are also claimed to have antibacterial, antifungal, and nematocidal properties. These all need to be verified through thorough investigations, and the potential for commercial products evaluated.

RESEARCH NEEDS

1. There is a need to determine the effects of the environment versus genetics on the various yield components in order to maximize production. Why do some trees begin to produce in 2–3 years, while others do not produce until 5–7 years after planting?

2. Superior genotypes need to be identified, to be used as mother plants to establish seed orchards, as sources of superior seed.

3. Germination and propagation methods need improvement.

4. Cultural practices need to be improved. Much has been done for forestry, and biomass pròduction, with little thought to fruit, seed and azadirachtin production.

5. The other active ingredients in neem extracts need to be identified and characterized.

6. Harvesting, handling, and extraction methods need improvement.

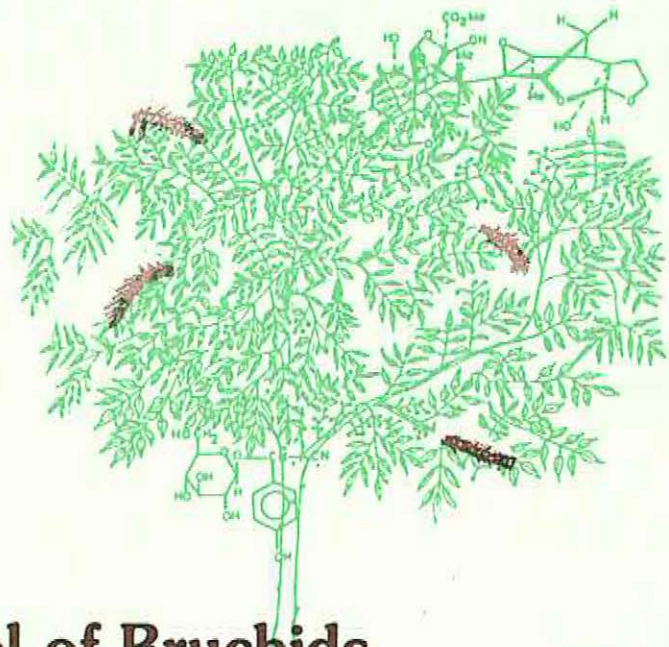
7. Protocols for processing of neem seed, including standardization of specifications for derivatives (oil, azadirachtin, etc.) are needed.

8. The effectiveness of neem extracts against virus, bacteria, fungi, nematodes, etc. and their potential uses in plant, animal and human health care must be elucidated.

9. The efficacy of various extracts and formulations as insecticides must be evaluated, including rates, and residuals.

10. Further investigations are needed into the use of derivatives as birth control agents.

11. Complete toxicological evaluations are needed of the various extracts. So far, extracts are cleared in the US only for plant care. They are not approved for animal and human health care.



Control of Bruchids and Weevils Using Traditional Insecticidal Plants from Sierra Leone

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Control of Bruchids and Weevils Using Traditional Insecticidal Plants From Sierra Leone

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INTRODUCTION

The cowpea (*Vigna unguiculata* L.) is a legume which forms an important part of the cropping system particularly in East and West Africa. There are two local varieties, the 'Tabe' and the 'Musaia' beans with brown and white testa coats, respectively. The growing and storage of this legume and certain cereals in Sierra Leone is entirely the business of the native farmer. Consequently, bruchid infestation of legumes, has continuously deprived the local subsistence farmer and his family of a vital dietary component, as well as a healthy cash return on seasonal harvests. The grains have a protein content of about 25% (Oyenuge, 1955). It is a very cheap source of protein for the rural population especially between harvest periods, in areas where alternative sources of protein are either scarce or expensive.

The use of synthetic insecticides against pests is though essential for the local subsistence farmer, yet prohibitive costs, inadequate handling facilities, and the fear of environmental pollution have compelled them to seek alternative methods of grain preservation. To control post-harvest infestation, local farmers mix dried plants with alleged anti-feedant/repellent properties with harvested grains intended as food, feed and seed. Losses, through insect infestation have reached alarming proportions, therefore, five plants from the Labiateae family and cloves *(Eugenia caryophyllata)* have been chosen for this scientific study using the pest target, *Callosobruchus maculatus* (L). Field studies are anticipated to determine whether the suspected terpenoid compounds involved can be further exploited to reduce stored grain losses.

OBJECTIVES

1. To evaluate the efficacy of *H. spicigera* and *E. caryophyllata* as insecticides against *C. maculatus* (L).

2. To determine the effective levels of application (EC_{50}) of *H. spicigeta* extract adequate enough to prevent infestation under natural conditions of temperature, pressure, humidity etc.

METHODOLOGY

Plants

Plants studied were *Hyptis spicigera* (Lam.), *H. sauveolens* (Poit), *H. lanceolata* (Poit), *H. pectinata* (Poit) and *H. afrorubens* (Lam).

Test Preparations

1. The aerial parts of *H. spicigera* were collected from three locations in Sierra Leone: (i) Musaia in the Kabala District; (ii) Batkanu (Port Loko District); (iii) Fintonia (O.K. National Park). Samples were maintained in the University Herbarium at Njala University College, Njala.

2. Samples of cloves (*E. caryophyllata*) were purchased from the local markets and dried.

3. Dried and finely powdered samples of the aerial parts (1.2 kg) of *H. spicigera* was extracted with acetone at room temperature for 10 days. After filtration, the crude extract was diluted stepwise after removal of pigments. Aliquots of 3, 10, 30, 100 and 300 µl of the resulting extract were diluted with solvent to a volume of 300 µl respectively. This amount of extract was observed to completely cover and adequately soak an amount of cowpea beans (circa 50) weighing approximately 10 ± 0.2 g.

4. The dried samples of cloves were finely powdered and the following portions accurately weighed out 0.3, 0.5, 0.7, 1.00, 1.2 and 1.4 g.

5. 50 beans weighing approximately 10.0 ± 0.2 g was used in all tests. Each test consisted of beans soaked in five different volumes of extracted solution. A solvent control and an untreated control were also conducted. Each test was repeated five times and the mean of results determined.

6. Six separate 20 g samples of beans were weighed and placed in different Mason jars.

Insect Rearing

The bean weevil under investigation was *C. maculatus*. Original samples were supplied by Rokupr Rice Research Station of Rokupr, Port Loko. These original weevils were raised in the laboratory in large jars on a diet of cowpea, *Vigna unguiculata* in an environmental chamber allowing normal conditions of breeding. After 28 days new adults emerged. For all tests, only adults of age 36 hours and under were used. After a period of 30 days all dead adults were removed from the chamber, the beans were then placed in special glass jars to await eclosion of the adults from pupae. All materials, i.e. beans for feeding, for testing etc. were kept in the chamber to maintain constancy of experimental conditions.

Insect Test Preparation

All insects used were below 30 hours after emergence. Remaining insects and frass were removed by sifting. New cultures were started with insects not used on tests. The insects were sexed by examination under a dissecting microscope (Howe and Currie, 1964; Raina, 1970; Southgate *et al.*, 1957).

Choice Testing

Glass vials of size 25 x 100 mm containing treated beans were arranged in a dessicator, or a suitable container e.g. fish tank, in concentric circles but equidistant from each other. Suitably sized tinfoil was cut to fit the dimensions of the vials. This was adhered to a piece of cardboard which fitted tightly around the circumference of the dessicator. The vials were made to fit snugly into the cardboard in an upright position in a random manner. About 100 unsexed insects were then placed in the centre of the dessicator and covered. A hole cut in the centre of the cover had been plugged with loose cotton wool.

No-choice Testing

30 insects of age 36 hr and less (15 males and 15 females) were placed into each vial and stoppered with a cotton wool plug.

Toxicity Test With Powdered Cloves

Each 20 g sample of seeds plus a weighed out portion of clove powder and ten pairs of weevils (males and females), constituted a treated unit.

Each treated unit was replicated four times. Mortality counts were taken after 24 hours.

Test Conditions

1. All tests were conducted in the environmental chamber.

2. The test vials were examined daily for adult mortality; some others were left for a period of 28 days before examination.

3. On the 29th day, all eggs laid in each vial were counted prior to new emergences using a fine brush and counting dial.

4. All eggs were returned to vials afterwards to await emergence of adults.

5. Every 2 days the adults emerging in each vial were counted and removed. These were later sexed to determine the pattern of male/female emergence.

RESULTS

1. A very high percentage of adults emerged over a period of 15 ± 2 days in all test samples with no difference in the time required for initial emergence. The male usually emerged earlier but the emergence pattern for male/female emergence was similar.

2. Again no remarkable differences were observed in adult mortality between controls and treated beans. Indeed, it appeared that the adults had a longer life-span on treated beans.

3. In all, no-choice and choice tests, increasing the concentrations of the *Hyptis* extract resulted in a very sharp decline in (a) oviposition (Fig.1) and (b) Percent emergence (no. of adults from pupae vs. total amount of eggs laid) (Fig. 2). (EC_{50} = 22 µl extract/g bean). These sharp declines commenced noticeably with concentrations above 30 µl/10 g treated beans.

4. Clove powder has showed a remarkable effectiveness as an insecticide against *C. maculatus* infestation. The 24 hr LC_{50} and LC_{90} values of 0.84 and 2.06 respectively, obtained from the graph of

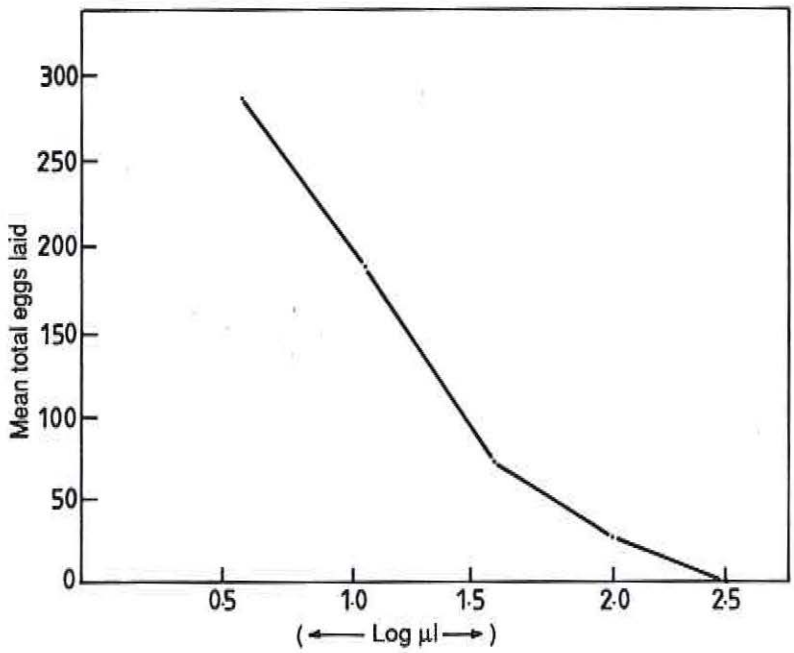

Figure 1. Mean total eggs laid on 3-choice tests trials of acetone (1g/ml) *Hyptis* added to 10 g beans.

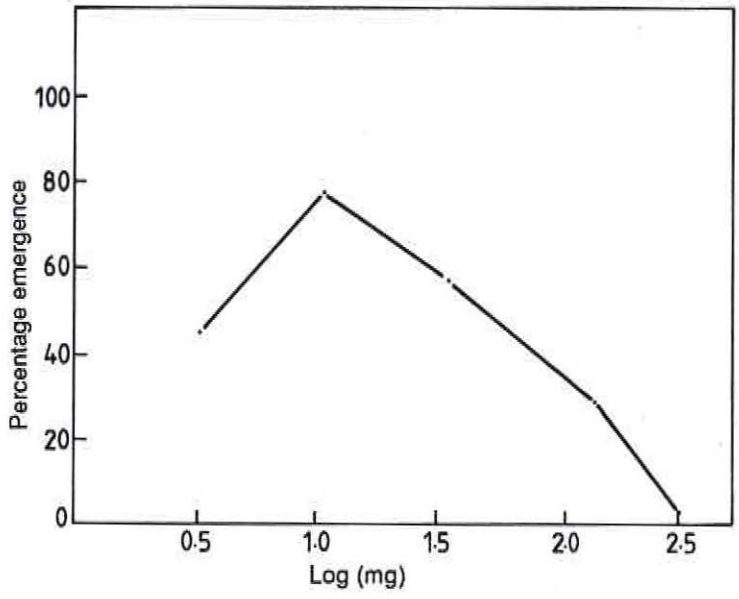

Figure 2. Mean percentage emergence of 3-no-choice trials of *Hyptis* extracts.

probit values of mean percent mortality/log concentration are indicative of this observation.

DISCUSSION

Eugenol from the oils of *C. sauve* and *E. caryophyllata* and a terpenoid fraction have been demonstrated to be a repellent against *Sitophilus zeamais* (Hassanali *et al.*, 1990). These active ingredients in *E. caryophyllata* can be assumed to be additively responsible for the toxicity of the clove powder against *C. maculatus*.

Further testing with the oil extracted from cloves is anticipated. Eugenol is known to be an attractant kairomone for several insect pests e.g. *Musca domestica* (Sharma and Saxena, 1974), the northern corn rootworm, *Diabrotica barberi* (Ladd *et al.*, 1983). *Hyptis specigera*, like *Hyptis sauveolens* and *Hyptis mutabilis* may contain, as active ingredients, a combination of terpenoid compounds (Rogelio and Mariano, 1988). The isolation, chemical characterization and structural elucidation of the active ingredients involved is also anticipated.

CONCLUSION

Further detailed studies of the insecticidal response of *H. spicigera* and *E. caryophyllata* to *C. maculatus* are anticipated, since this preliminary study has indicated the potentials of these botanicals as pesticides. With further research into similar uses of botanicals and training of local farmers on the socio-economic aspects of these uses, the alarming destruction of stored grains by stored grain pests can be reduced and the process made extremely cheaper for the farmer.

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Natural Products as Pesticides in Cameroon

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Natural Products as Pesticides in Cameroon

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INTRODUCTION

The tropical rainforest of Cameroon is of great interest from the point of view of phytochemical ecology. It is rich in plant species which have adapted in a co-evolutionary fashion not only to climatic factors but also to the complex interactions occurring between plant and animal, plant and micro-organisms or plant and plant in the tropical environment (Koshimizu *et al.*, 1985).

Secondary plant metabolites such as alkaloids, flavonoids, terpenoids and naphthoquinones etc. have been shown to have a significant role in these complex interactions (Koshimizu *et al.*, 1985). The study of such interactions between bioactive metabolites and other living organisms could provide new information on natural pesticides and help in the conservation of natural communities. Due to the varied climatic zones found in Cameroon, the study was also extended to the savanna regions. About 85% of the world's production of pesticides is used in industrialized countries. However, toxicity from pesticides is 13 times higher in Third World countries as compared to industrialized countries (Gilles, 1989). Over 2 million cases of intoxication by pesticides are estimated to occur yearly resulting in at least 40,000 deaths. Even some chemical compounds synthesized to have structures like their natural analogues (e.g. pyrethrinoids) have recently been found to be toxic (Gilles, 1989).

In a developing country like Cameroon, the use of synthetic pesticides came as a relief to farmers. It was made affordable to them through Government subsidies and crop yields increased. However, over 1000 cases of insecticide-linked deaths have been reported yearly, the causes of which are linked to failure of carrying out proper procedures for storage, handling, use and user protection.

Because of these problems, and the ever increasing ecological concerns and financial costs of imported pesticides, the need to search for natural products that can take their place has become acute. An approach that has been used for the discovery of higher plants with

active secondary metabolites, and consequently those with pesticidal properties, is to evaluate extracts from medicinal plants that are well known and used economically in folk medicine. In Cameroon, wood ash and some indigenous and/or introduced plants (garlic, pepper, paw-paw, lemon grass, *Ocimum* spp. etc.), are used to protect field crops as well as stored grains and tubers against pests. *In vitro* tests on some of them (Nankam and Njehoya, 1988) showed activity against spore germination of *Collectotrichum coffeanum*, a pathogen causing coffee - berry disease.

This approach has the advantage that human experience in the use of these plant materials is well established. What is lacking is scientific confirmation of the efficacy, toxicity and mechanism of action of the substances employed.

Quick and simple bioassays carried out on crude plant extracts can give an idea of the presence or absence of any biological activity, and may give a useful direction to further investigations.

Another successful approach has been the study of natural products, particularly allelochemicals, which play key roles in the chemical defense of plants. A prerequisite is careful observation of the ecological environment of such plant species.

METHODS AND RESULTS

Plant parts collected were quickly sun-dried and taken to the National herbarium for identification (Table 1). They were ground and each plant extracted with methanol in a continuous soxhlet apparatus for 3 days. Each extract was adjusted to 20 ml and then used in bioassays (Table 2).

Plant Germination and Growth Test

On a Pyrex glass dish (70 mm dia., 100 mm deep) absorbent cotton (50 x 50 mm) was placed, and 0.1 ml of an emulsified solution (1 part Tween 80 : 10 part acetone), containing a certain amount of the test compound and 10 ml of water were added. Usually the test was started at a concentration of 1000 ppm. Seeds of the test plant (three in cucumber, *Cucumis sativas*, seven in barnyard grass, *Echinochloa crus-galli* and 30 in mustard, *Brassica juncea*) were sowed on the cotton and incubated at 27–28°C under white fluorescent light. After 10 days, the germinated seedlings were observed and evaluated for germination and growth (Table 3).

Table 1. Plants of interest at Mwini and Yaounde (in the tropical rainforest)

Plant	Family	Tropical use
Pachypodantium staudtii	Annonaceae	Louse expellent
Xylopia quintasii	"	Stomach medicine
X. aethopica	"	Animal antifeedant
Meiocarpidium lepidotum	"	Worm expellent medicine
Alstonia boonei	Apocynaceae	Malaria treatment
Strophantus gratus	"	Arrow poison
Emilia coccinea	Asteraceae	Antidote
Scorodophioeus zenkeri	Cesalpinaceae	Foodstuff for flavouring
Hoplestigma klaineanum	Hoplestimataceae	Animal anti-feedant
Endodeama calophylloides	Hypericaceae	Liver medicine
Holopegia azurea	Marantaceae	Antidote
Staudtia stapitata	Myristicaceae	Medicine for injuries
Lophira alata	Ochnaceae	Medicine for toothache
Pterocarpus soyauxii	Papilionaceae	Timber
Maesopsia eminii	Rhamnaceae	Medicine for lumbago
Baillonella toxisperma	Sapotaceae	Allelopathic
Duhoscia macrocarpa	Tiliaceae	Animal anti-feedant

Table 2. Bioactive plants selected through bioassays

Plant	Family	Activity*	Traditional use
Pachypodantium staudtii	Annonaceae	B	Louse expellent
Anonidium mannii	Annonaceae	P	Randomly collected
Xylopia aethiopica	Annonaceae	P, B	Animal antifeedant
Strophantus gratus	Apocynaceae	B	Arrow poison
Guibourtia ehie	Cesalpinaceae	P	Randomly collected
Guibourtia tessmannii	Cesalpinaceae	A	"
Tetrabertinia bifoliolata	Cesalpinaceae	P	"
Mareyopsis longifolia	Euphorbiaceae	A	"
Lophira alata	Ochnaceae	A	Medicine for toothache
Rothmannia hispida	Rubiaceae	P	Randomly collected
Baillonella toxisperma	Sapotaceae	P	Allelopathic
Duboscia macrocarpa	Tiliaceae	B, A	Animal anti-feedant

*A, antibacterial activity; B, inhibitory to brine shrimp development; P, plant growth inhibitory.

Table 3. Plants which exhibited inhibitory activity on plants growth

Plant*	Part	*E. crus-galli*		*B. juncea*		*C. sativas*	
		stem	root	stem	root	stem	root
Trichoscypha acuminata	A	+	–	+	+	–	+
Meiocarpidium lepidotum	A	–	+	+	+	–	–
Pachypodanthium staudtii	B	+	++	+	+	+	++
Xylopia aethipica	B	+	++	++	++	+	+
X. quintasii	B	+	+	++	++	+	–
Strophantus gratus	A	+	+	+	+	–	–
Spathodea campanulata	A	++	++	+	++	–	++
Stereosparmum acuminatissimum	A	+	++	+	++	–	++
Hymenostegia afzelli	A	–	–	–	++	–	+
Tetraberlinia bifoliolata	A	+	+	–	+	+	+
Acalypha wilkesiana	A	+	++	++	++	+	+
Breynia nivosa	A	–	–	++	++	+	+
Icacina claesensii	A	+	++	+	+	+	++
Memecylon arcuatomarginatus	A	–	–	+	++	–	+
var *simul*	S	+	–	–	±	+	++
Baillonella toxisperma	L	–	–	–	++	+	++

*A, aerial part; B, bark; S, stem; L, leaf; –, not active ±, slightly active; +, active; ++, strongly active.

Animal Anti-feeding (Brine Shrimp Development)

For the inhibition of brine shrimp *Arsenia salina* development, dried eggs obtained from Pet Drugs Co. Ltd. Tokyo, Japan were used. The eggs (0.3 g) were incubated in a 2% NaCl solution (300 ml) for 48 hr. One ml of the solution containing about 100 hatched nauplius larvae was pipetted out and introduced into a cylindrical vessel (15 mm in dia. 20 mm deep), to which 50 µl of the extract solution was added. Mortality of the nauplius larvae was observed over 6 hr, and compared to that of a control experiment without plant extract. Four species showed anti-feedant activity (Table 2).

Antibacterial Activity

A paper disc (8 mm dia.) was dipped once in the extract solution, placed on an agar plate and allowed to stand overnight. The antimicrobial zone (the clear zone around the disc) was then measured. Four species showed antibacterial activity.

Anti-fungal Test

The emulsified aqueous solution containing the test compound was sprayed on leaves and shoots of plants which are usually vulnerable to particular fungi. The leaves were allowed to dry then sprayed with spores of the specific fungi known to infect the plant. The individual plant was then incubated under suitable conditions for the growth of the fungus. The results are given in Table 4.

Table 4. List of plants which exhibited anti-fungal activity

Plant*	Part	Activity	Fungus
Afrostylax kamerunensis	A	+	*P. infestans*
Baillonella toxisperma	L	+	*E. graminis*
Beilschmiedea klainei	A	+	*Botrytis cinerea*
Desmostachys tenuifolius	A	+	*E. graminis*
Diospyros obliquifolia	A	+	*E. graminis, P. triticina*
Guirbourtia tassmannii	A	+	*E. graminis*
Hypodaphnis zenlceri	A	+	*P. infestans*
Memecylon arcuatomartion var. *simul.*	A	+	*E. graminis*
Onchthocosmus calothyrsus	A	+	*P. triticina*
Porterandia ladantha	A	±	*E. graminis*
Pterocarpus soyauxii	A	+	*P. infestans*
Rauwolfia vomitoria	B	+	*Phytophthora infestans*
Schumnniophyton magnificum	B	+	*P. triticina*
	S	++	*E. graminis*
Spathodea campanulata	A	+	*E. graminis*
Staudtia stipitata	B	±	*P. oryzae, C. sasakii*
Strophantus gratus	A	+	*E. graminis*
Trichoscyptia acuminata	A	+	*Erysiplie graminis*
Villosa	A	+	*Puccinia triticina*
Xylopia quintasii	B	+	*P. cubensis*

*A, aerial parts; B, bark; S, stem; L, leaf; ±, slightly active; +, active; ++, strongly active. No plants which had been selected at Mwini to test for antibacterial activity exhibited the anti-fungal effect.

Herbicides: The Allelochemic Effect?

One of the plants studied, *Baillonella toxisperma* (Sapetaceae) is a high tree which showed a characteristic phenomenon in the forest. Around the parent tree about 50 m in height, only its young trees (with an average height of 1.7 m) were growing. Their growing sphere was coincident with that of the crown of parent tree. This phenomenon might be characterized as an allelopathy which should be mediated with some specific constituent excreted from the tree.

Spectral and chemical data suggest that the active compound is a pyrimidine nucleoside. This compound was shown to inhibit cucumber

root growth completely at 50 ppm and by 50 percent at 25 ppm and to inhibit the growth of radish by 50% at 50 ppm (Koshimizu *et al.*, 1985).

Molluscicides

In Cameroon, 75% of the Northern population suffer from the vector-bone disease bilharzia, transmitted by snails (infected with *Schistosoma mansonii*).

From mollusc sampling (following network in UNEP 1985), it appears that extracts of four plants at very low concentrations may have the potential to hold the *Schistosoma* larvae and the intermediate host snail completely (Mokondjie, per. comm.).

The four plants studied were *Milletia* spp. (Leguminoceae), *Balanites aegyptica* (Zygophyllaceae/Balantinaceae), *Balanites wilsoniana* (Zygophyllaceae/Balantinaceae), *Phytolacca dodecondra* (Phytolaccaceae).

Nematocides

The plant *Chenopodium ambriosicides* (Chenopodeceae) was collected from the savanna region of North Cameroon. The volatile oil from the seeds has been used in folk medicine to expel round worms and hook worms. The ascaridol content of the oil was assayed using standard methods. A 5% w/v solution of the oil in 90% acetic acid was prepared. 5 ml of this solution was added to a previously cooled mixture (3°C), consisting of 3 ml 85% aqueous solution of potassium iodide, 5 ml of hydrochloric acid and 10 ml of glacial acetic acid. This solution was then placed in a stoppered tube and set aside for 5 minutes in a cool place.

The liberated iodine was titrated with N/10 sodium thiosulphate. At the same time, a control test (blank) was carried out without the oil. The difference between the two titrations represented the iodine liberated by ascaridol. Each ml of N/10 sodium thiosulphate was equivalent to 0.0663 of ascaridol. Chenopodium contained 1% of volatile oil which consisted mainly of 70% ascaridol. Other plants studied and found to be effective against round worms are *Canthium* spp., *Borhevia* spp. and *Carica papaya* (unripe fruits).

Insecticides

Of all the types of pesticides studied we were most interested in the search for insecticides from plants due to the following reasons:

(a) the final elimination of many parasitic diseases will depend on the destruction of insect vectors. Many tropical diseases like filaria which causes blindness and elephantiasis (a condition characterized by a massive enlargement of the scrotum and one or both legs), are transmitted by insects. Hence, there is a growing awareness in the use of insecticides as a measure of prevention by destroying insect vectors.

(b) From the agricultural point of view, insects are constraints to food production and crop protection can only be achieved by the elimination of insects as well as their larvae which destroy food crops.

(c) To replace synthetic insecticides (though efficient as contact and systematic poisons on pests) which are toxic and lethal to man and animals and remain in the atmosphere for hours, days and even weeks after spraying, causing environmental pollution. Recently, some researchers have shown concern about some pathogens (Nankam *et al.*, 1988).

(i) *Collectotrichum coffeanum*, which cause coffee berry disease.

(ii) *Phytophthora infestans* which cause late blight in potato and tomatoes (two important cash crops in the country).

With the current economic situation in the country, state subsidies for pesticides have dropped drastically.

It was, therefore, necessary to exploit the rich flora of Cameroon (which englobes over 2500 species of plants), to search for natural pesticides from plants which are less toxic and readily biodegradable (Fig.1). Although alkaloidal compounds have been isolated from several plants with insecticidal properties, they are as a rule, still toxic to warm blooded animals, e.g. nicotine from tobacco plant (*Nicotiana tobaccum*) and furanoquinoline alkaloids from *Teclea ouabanguiensis* (Ayafor *et al.* 1982).

The toxicity of many other insecticidal compounds obtained from plants e.g. limonoids (Ayafor *et al.*, 1986) still need to be studied.

The pyrethrins and rotenones (Fig. 2) are both of natural origin and have up till now been proven to be devoid of toxicity.

The pyrethrins are esters and liable to cleavage. They are obtained from the flower heads of several species of cultivated *Chrysanthemum*. The rotenones are isoflavone derivatives. They are more stable. They have been isolated from several species of *Tephrosia* and *Derris* plants which are found in Cameroon. Resistance of insects to rotenones has not yet been observed though large quantities are necessary for permanent kill. However rotenone can cause skin irritation.

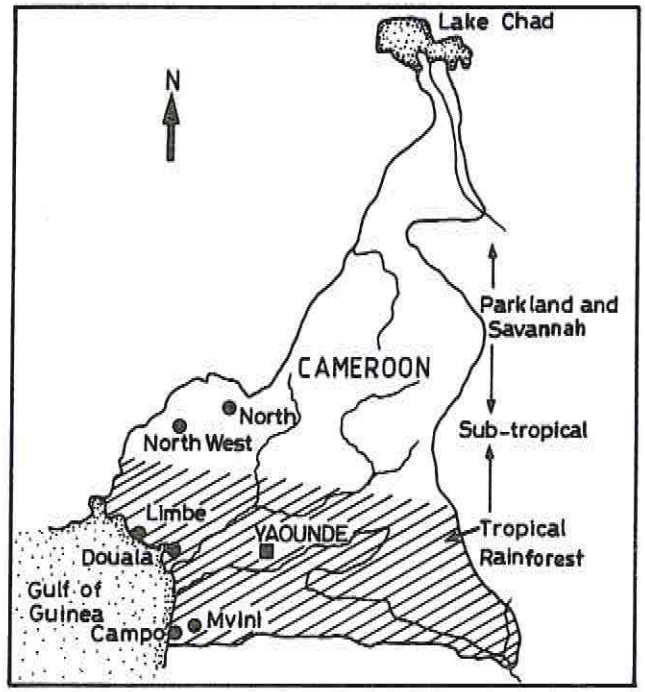

Figure 1. Map of Cameroon indicating sampling areas.

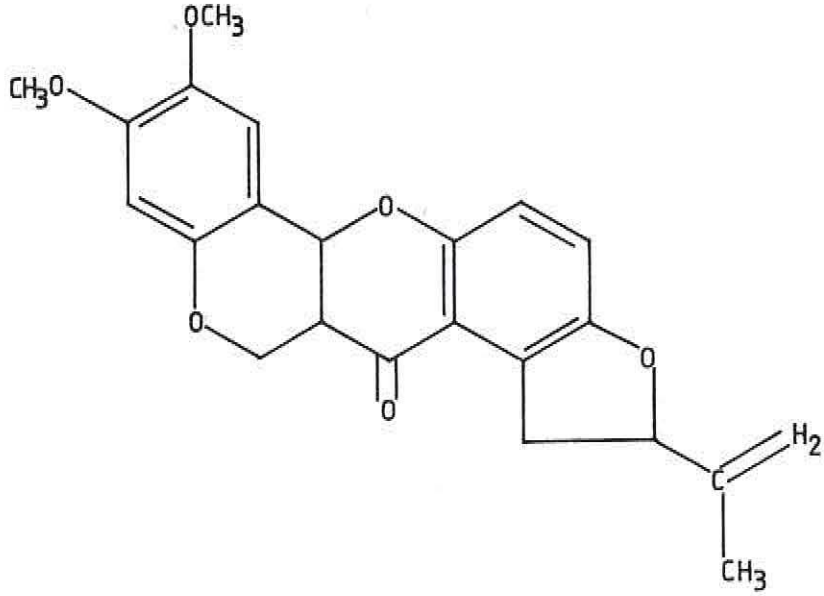

Figure 2. Rotenone flavonoid compound, a compound obtained from *Tephrosia virginiana* with insecticidal property.

As an observation from some farmers in the North West Province of Cameroon, the leaves of *Ocimum suave* and *Tephrosia alata* , have been used to protect stored grains from insect devastation.

In other cases, the seeds and roots of the plants *T. virginiana* (Devils shoestring) and *T. toxicaria* were ground, mixed with oil, paraffin oil and emulsifying agents and perfuming agents. They were excellent as household, cattle, garden plant and sprays without any harmful effect on man or animals.

The plants activities were suggestive of anti-feedant and or insecticidal properties of the rotenone type.

Anti-feedant Effect

The insects avoidance of the stored grains covered by *Ocimum* leaves is suggestive of a natural defense mechanism (i.e. the escape from the ingestion of toxic substances or allelochemics that would destroy it).

For the study of insecticidal properties, a weighed quantity (20 g) of the seed or root was extracted with water (250 ml) for 3 days to obtain the total extract. Secondly a weighed quantity of seed or root (20 g) was also extracted with solvents (250 ml) each of increasing polarity — ether-methanol-water.

Insecticidal Activity

The total extract as well as the individual extracts obtained above were used in increasing concentrations against the following insects: (Enyong, 1989)

- *Simulium damnosum* complex — blackfly
- *Musca domestica* — housefly
- *Zonocerus variegatus*
- *Macrotermes* species
- *Anopheles gambiae*
- *Sitophilus* species on cereals.

The ether extract as well as the total extract were found effective. The extracts were observed (Enyong, 1989) to give a quick knock down to all the chewing and sucking insects. The extracts gave complete kill at about 2% concentration each. However recent studies from some workers (Kaposki, 1990) have also shown activity against mites and ticks (ascaridol). Therefore, several species of *Tephrosia* were studied for their rotenone content and their total ether-soluble or acetone-soluble extractive.

The ether extract was cleaned through a florisil column and eluted with hexane, ethyl acetate 9:1 which gave pure rotenones and further elution with hex: ethyl acetate 4:1 gave the mixture of the other compounds mentioned. The pure rotenone as well as the total acetone-soluble extractive were individually found to be active against all the same insects mentioned earlier (Enyong, 1989). All the *Tephrosia* spp. contained rotenone (Fig. 2), degueline and toxicarol compounds which are all structurally related and have insecticidal effects as the roots and rhizomes of *Derris eliptic*.

The seeds and roots of all *Tephrosia* spp. in each case gave 3% rotenone and over 10% acetone-soluble extract. *Derris* roots and rhizomes gave over 8 % rotenone.

Indigenous farmers of the North West prepared the mixture of the extract in oil or emulsifying agents prior to application on cattle, to reduce irritation. Further investigations to reduce irritation properties of rotenone evaluation of insecticidal properties of *T. virginiana* extract are envisaged.

White mice (Sprague type) were fixed on a support and were placed individually for 24 hours in a cage each containing 50 unnourished (for 48 hours) malarial mosquitoes (*Anopheles gambiae*) produced by the insect breeding section of the Medical Research Station, Kumba. Surrounding temperature of the laboratory was 24 ± 2°C. A deponent cage and two cages with individual test extract were used. Extracts were sprayed into cages (using crude buckets, a technique used by farmers), while protecting eyes and noses. The results showed that the mosquitoes took blood meal (0–24 hr) from mice in deponent cage whereas no mosquito fed from mice in extract-treated cages (ether or aqueous).

CONCLUSION

The tested products in accordance with the test protocol of our laboratories, have shown a very important insecticidal activity. In the experimental cages, no mosquitoes bit a mouse after a contact period of 12 hours. In contrast with the deponent cage, 96 % of the mosquitoes were engorged after the same contact period.

After 12 hours of contact, the two mice of the experimental cages did not show abnormal mortality. The morning of the experiment, they were nourished on a special meal. The tested products showed only insecticidal activity. The mechanism of action will be studied further.

Larvicidal Activity

Three one cubic metre tanks were each filled with water and about 1000 larvae of the mosquito *Anopheles gambiense* carefully introduced into each tank and covered with a fine plastic material furnished with a suitable device to allow the constant supply of oxygen into the tanks. No larvae were added to the last tank. The larvae showed sudden detachment from surface, dropped to bottom, and died instantly (Enyong, 1989), in the tanks into which aqueous and ether extracts were introduced.

Anti-viral Activity

Further to the study on the bioassay analyses of the plants, some researchers (Koshimizu *et al.*,) believed that *Lophira alata* (Family Ochnaceae) contains a flavonoid compound that could be active against some viruses. Also *Angylocalyx talbotic*, a plant found in the Korup region of Cameroon has been strongly suggested as an effective antiviral agent.

Isolation and Characterization of Compounds

As a step further, some of the compounds have been isolated and characterized using standard chemical and spectral techniques (Fig. 3) (Fomum *et al.*, 1986). The ground dried plant part was weighed and extracted in a continuous soxhlet apparatus with solvents of increasing polarity. The extracts were then concentrated *in vacuo* and each extract placed on an absorbent material (silica gel G 60) deposited on a glass column and compounds separated using a suitable mixture of organic solvents. The structures of the pure compounds were elucidated and characterized using IR, UV, MS, and NMR spectral techniques and chemical correlations (Fomum *et al.*, 1986).

Erythrina sigmoidea

Three new flavonoid compounds were identified and their antibacterial effects confirmed (Biyiti *et al.*, 1988). From *Diopsyros* species, naphthoquinones (Fig. 3) (Waterman *et al.*, 1979) were obtained which exhibited anti-fungal effect (Koshimizu *et al.*, 1984). These compounds have also been implicated in the resistance of the red and hard timber (of the Ebeneceae family) to destruction by termites.

Mode of Action

The mode of action of rotenone on all sucking and chewing insects is

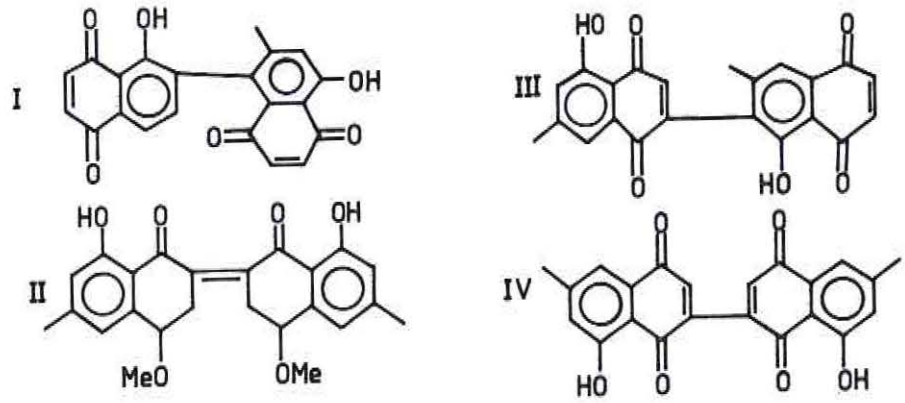

Figure 3. Hydroxy naphthoquines obtained from several species of *Diospyros* (Fam. Ebeneceae) with antifungal effect.

still not clear, but it would appear that the insecticide could penetrate the cuticle of the sucking insect. It is then transported through the blood stream to the gut where it precipitates the protein of the insect.

In the case of chewing insects, the insect ingests the insecticide which is transported directly through to the gut of the insect where the protein in the gut is precipitated. The presence of the Carbonyl group -C=O, is largely implicated in this type of action.

Another mechanism of action is linked to the absorption and transportation of rotenone to the nervous system of the insect to cause paralysis. A further study to estimate the activity of cholinesterase after application of extracts is recommended.

DISCUSSION AND RECOMMENDATIONS

The compound rotenone from the seed and roots of *T. virginiana*, and the roots and rhizomes of *Derris* spp., has been shown to possess insecticidal effects. Preliminary bioassays on extracts of several plants collected from the TRF and savanna regions respectively have shown that these possess one or more pesticidal activity.

Ocimum sauve is known to contain eugenol (Trease and Evans, 1978) and is used in traditional medicine to deter insects from stored grains (repellent effects on insects). The above results justify the increasing credibility on local plants as potential therapeutic agents, and suggest that there could be basis for the use of these plant materials in traditional methods of plant and animal protection.

Plants in the TRF showed selective action against other bacteria and fungi. However, those which were found active against bacteria had no activity against fungi. Results from the study demonstrated a possible scientific rationale for the use and preparation of a single plant extract or mixtures of plant extracts in the production of natural insecticides. These results confirm the richness of the Cameroon flora in plant species and the need for serious studies in production of insecticides at industrial levels from plants, especially from fast growing shrubs like the *Tephrosia* species.

There is the immediate necessity for 'conservation strategy' which includes preservation of genetic diversity, maintenance of ecological processes and ensuring that any utilization of species is sustainable.

ACKNOWLEDGEMENT

I wish to thank the National Research Council for the financial assistance and the incentive given to me to present this review as a preliminary and comprehensive data summed up to be helpful for subsequent surveys in the future.

Special thanks go to Prof. Jato Johnson for making the short term collaboration with Prof. Koshimizu's team possible. The effort of Prof. Koshimizu to examine further biological activities in detail cannot be overemphasized.

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Plants Used for Control of Insect Pests on Stored Grains in Cameroon

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Plants Used for Control of Insect Pests on Stored Grains in Cameroon

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INTRODUCTION

Stored products may be destroyed in storage by insects, fungi and vertebrate pests, insects being the most important. These pests destroy about 1/3 of the world's yearly harvest, valued at about 75 billion U.S. dollars (Marini-Bettolo, 1977).

Crop losses in store are more severe in humid tropical countries. Caswell (1968) estimated an annual loss of 24,000 tons per 1 million of stored pulses in Nigeria, because of infestation by the common African pulse weevil, *Callosobruchus maculatus* F. The situation may be the same in Cameroon, where *C. maculatus* and *Sitophilus zeamais* Motsch, respectively, devastate stored pulses and maize. In the northwest province of the northwest highland savanna zone of Cameroon, the FAO (1981) has estimated that losses in maize grain due to insects and moulds are between 12 and 44% during the first 6 months of storage. It is, therefore, necessary to reduce such high losses by controlling the pests on the stored products.

In Cameroon, casual field observation for the past few years has shown that some farmers and many middle men who sell stored products such as shelled maize, beans, cowpeas and kola nuts (*Cola nitida* and *C. acuminata*) commonly treat them with hazardous pesticides such as Thiodan, DDT dust or pirimiphos-methyl, at relatively high doses. Many of these hazardous pesticides are toxic to man and other non-target organisms, enhance the development of resistance in some stored-product insect pests and cause environmental pollution. These disadvantages necessitate the search for allelochemicals or 'fourth

generation' insecticides. These are of plant and animal origin and described by Rosenthal (1986) as being less toxic to man, readily biodegradable, suitable for use by small scale farmers and yet capable of protecting crops from attack by a diversity of insects.

Many African plants are potential sources of 'fourth generation' pesticides and have been shown to contain anti-feedant, repellent or insecticidal compounds that enable either the crude plant materials or the extracted active compound to protect field crops or stored products. Anti-feedants have been extracted from African plants such as *Warbugia stillmanii* and *W. ugandensis* (Kubo *et al.*, 1976), *Azadirachta indica*, the neem tree, *Melia azadirach* whose leaves are used also for control of insects on stored grains by East African farmers (Kubo and Nakanishi, 1977) and *Tephrosia elata* (Bentley *et al.*, 1987). Insecticidal compounds have been extracted from plants such as *Teclea oubanguiensis* (Ayafor *et al.*, 1986), *Lippia adoensis* and *Piper guineense* (Olaifa *et al.*, 1987). In spite of the paucity of information on pesticidal plants in Cameroon, it is known that many farmers in the northwest highland savanna zone traditionally use plant materials to control insects on maize, beans and cowpeas stored in traditional bamboo cribs or calabashes. Many of the plants used by the farmers have not been identified and documented, so there is no base line information on potential 'fourth generation' insecticidal plants. The main objectives of this study were, therefore, to survey, identify and document the plants used in controlling insects on stored maize, beans and cowpeas in the major grain producing areas of the northwest highland savanna zone of Cameroon, and also to document how the plants are used in the traditional storage structures.

MATERIALS AND METHODS

The ethnobotanical survey was conducted from July 1986 to May 1987. The three sites chosen in the study area were Awing (Bamenda district), Bamunka (Ndop district) and Oku (Bui division). These sites were chosen because they are all located in the major grain producing centres of the zone.

Information on plants used in the control of insect pests on stored maize, beans and cowpeas was obtained from elderly indigenous farmers. In each site, the traditional rulers and the local agricultural authorities were asked to recommend farmers for interview. Those recommended were knowledgeable in the traditional use of plants for insect control on stored grains. A total of 30 farmers were interviewed in Awing and 20 each in Ndog and Oku.

The interviewed farmers in each study site revealed the plants used in stored grain protection and demonstrated their use in the indigenous grain storage structures ('Banda', raffia bamboo cribs, calabashes, tree-bark containers or kerosene tins). Some of the plants used by farmers at the different sites were identified on site in the field by a systematic botanist. Where this was not possible, specimens (branches, shoots, leaves, flowers and stem bark) were collected and later identified at the National Herbarium in Yaounde, Cameroon. Both the vernacular and botanical names of identified plants were recorded, as well as the major storage structures from each study site.

RESULTS

Plants Used for Control of Insect Pests on Stored Grains

The indigenous plants commonly used in the three study sites for the control of insect pests on stored maize, beans and cowpeas are listed in Table 1. The use of plant materials for the control of stored pests in Awing, Ndop and Oku is a tradition which has been passed on for generations, although the botanical identity of these plants and their methods of use in the storage structures have not been documented.

For some of the plants, Table 2 presents information from the literature on their active compounds, the plant parts from which they were extracted and their probable action on insect pests. Many of the plants used for protecting stored grains in the study area appear to have either a repellent, anti-feedant or insecticidal effect on stored-product insect pests.

Methods of Utilization of Plant Materials

Usage in kitchen platforms ('Bandas') at Ndop: Leafy branches of *Celtis african, Teclea grandifolia* and *Cupressus* spp. were used either individually or combined in unspecified proportions for protecting stored maize in kitchen platforms which were usually situated above the cooking fire place in the firewood kitchen. The leafy branches are collected fresh and divided into two equal fractions. One fraction was scattered over the surface of the platform on which maize will be stored, using about 1 or 2 leafy branches per square metre. Dried, husked corn ears were then spread about 50 cm thick on top of the branches. The remaining branches were then spread uniformly on the layer of stored maize. The maize was left in this condition throughout the drying and storage period of 8–10 months. The leafy branches were usually not

Table 1. Identified plants and their parts used for the control of insect pests on stored grains in Awing, Ndop and Oku

Identified plants	Location of use and vernacular names of plants	Plant parts used	Protected stored product
Alingium chinense (Low) Harm Alangiaceae	Oku 'Febom'	Leafy branches and bark	Mainly maize ears
Celtis africana Burman fil	Ndop 'Nyonyuy'	Leaves and stem bark	Maize
Clausena anisata Will. Ex. Benth. Rutaceae	Oku 'Ey-mphy' Ndop 'Ngonyuy'	Leafy branches	Maize ears, beans and cowpeas
Cupressus spp. Cupressaceae	Awing 'cypress' Ndop 'cypress' Olu 'cypress'	Leafy branches " "	Maize, beans and cowpeas
Elaeis guineensis Jacquin Palmae	Ndop 'Tang'	Extracted edible oil	Beans and cowpea seeds
Ocimum sauve Wild Labiatae	Awing 'Ntsigne'	Leafy branches	Maize ears, beans and cowpeas
Capsicum frutescens Solanaceae L.	Awing 'Senteh'	Fruits	Beans and cowpea seeds
Teclea grandifolia Engl. Rutaceae	Ndop	Leaves	Maize

changed during this period, during which the maize was gradually used by the family.

Usage in air-tight storage structures : Beans and cowpeas were treated with palm oil at the rate of 1 table spoonful per 1 kg of seeds. The treated seeds were put into air-tight containers such as calabashes, tree-bark containers or kerosene tins, which were then sealed. The seeds were stored for a period of 6–8 months with little or no insect infestation. In Oku, the bases for the storage calabashes, tree-bark containers or kerosene tins were first lined with leafy branches of

Table 2. The chemical composition and action of some plants used for control of stored product insect pests in the N.W. highland savanna zone of Cameroon

Identified plants	Identified active compounds	Plant parts	Action of compound on insects	Source of information
Alangium chinense	Anabasine	Leaves & bark	Insecticidal	Shi-Dawen *et al.* 1983
	Ementin alkaloids	Leaves & bark	Insecticidal	Wolfgand *et al.*, 1984
Clausena anisata	Clausanitine and ananisatine	Stem, bark & roots		Okorie, 1975
	Alkaloids = Mupamine	Leaves	Insecticidal	Mester, 1983
	Imperatorin and Xanthoxyletin	Leaves	Anti-feedants	Gebreyesus and Chapya, 1983
	Anisocoumarins A,B,C, and D	Stem, bark & roots	–	Ngadjui *et al.* 1989
Elaeis guineensis	Triglycerides & Oleic acid fractions from edible oil	Edible oil from fruits	Insecticidal	Hill and Schoonhoven, 1981
Ocimum suave	Eugenol (oil)	Leaves	Repellent and Insecticidal	Chogo and Crank, 1981
Teclea grandifolia	Alkaloids	Leaves	Insecticidal	Ayafor *et al.* 1982
	Limonoids	Leaves	Repellent	Kubo, 1983 Ayafor *et al.*, 1986

Cupressus spp., *Clausena anisata* or *Alangium chinense* before the containers were filled with beans or cowpeas seeds. A combination of two or three plant species was occasionally used. The mouth of the filled container was usually plugged with the same plant material before it was sealed for a storage period of 6–10 months. In Awing, threshed beans or cowpea seeds were mixed with dried group *Capsicum frutescens* before being stored in the air-tight containers.

Usage in raffia/bamboo cribs at Awing and Oku : The people of Awing and Oku used similar storage structures (raffia or bamboo cribs, or air-tight containers) and similar techniques in protecting stored grains from insect pests with plant materials. However, the two groups of people used different plant species (Table 1). The people in Awing used leaves and leafy branches of *Ocimum sauve* and *Cupressus* spp., and ripe fruits of *C. frutescens*, while those in Oku used leaves and bark of *Alangium chinense* and leafy branches of *C. anisata* and *Cupressus* spp.

The farmers at both sites usually disinfested the bamboo cribs by thoroughly cleaning the inside and outside, then putting 6–9 leafy branches or bark of the desired plant into the cribs and sealing them. The cribs remained sealed with the materials inside for a period of 3–5 days, and then filled with ears of dehusked maize.

When filling the cribs with maize, the floor was first layered at the centre with 4–5 fresh leafy branches or bark of the desired plant. The crib was filled (about 1/3) with maize ears and the same quantity of plant material laid on top. This process was continued until the crib was filled. The last layer of maize was usually covered with a last layer of the plant material. A total of 4–6 layers of the desired plant material were used in cribs of up to 1.8 m height. The cribs were sealed for the storage period of 6–10 months, during which the maize was periodically used by the family. The plant materials were usually neither replaced nor removed from the cribs while the maize was used up.

In Awing and Oku, plant materials of one species were utilized alone or combined with those of another species. For example, in Oku, leafy branches of *Cupressus* spp. often used alone, were occasionally used with leafy branches of *C. anisata*. When used in combination, 4–5 leafy branches of each species were put at each level of the bamboo crib. In Awing, a combination of leafy branches of *O. sauve* and *Cupressus* spp. was utilized. Farmers in Awing and Oku believed that a

combination of plant materials was better in protecting their stored maize from insect infestation.

DISCUSSION

This survey revealed that farmers in the N. W. highland savanna zone of Cameroon use diverse plant materials from a variety of species to reduce insect damage on their stored maize, beans and cowpeas. The degree of damage reduction attributable to the use of the plant materials and their mode of action are not known.

In conclusion, many of the plant species observed to be useful in protecting stored products in the northwest highland savanna zone of Cameroon are potent sources of less hazardous compounds for insect control compounds that require detailed studies and exploitation.

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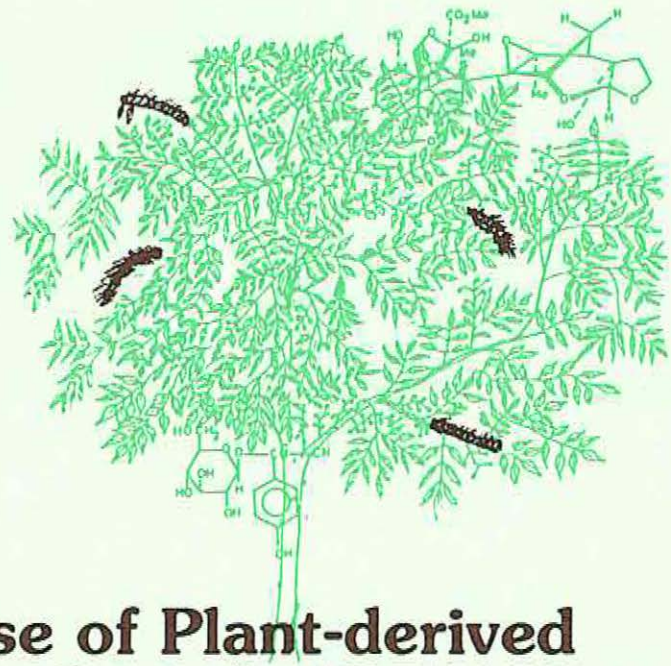

The Use of Plant-derived Anti-feedants in Pest Management in the Sudan

11

A.M. Zein El Abdin

The Use of Plant-Derived Anti-feedants in Pest Management in the Sudan

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INTRODUCTION

The importance of natural and inorganic pesticides is rather limited compared to the new synthetic ones. These synthetically produced pesticides are still the principal means of crop protection. They are valued for their uniform and rapid effectiveness against target pests, ease of storage and use and relatively long shelf-life under proper stage conditions. However, their use is accompanied by some major drawbacks such as toxicity to non-target organisms, development of insect resistance, insect resurgence, destruction of natural enemies and the environmental contamination with the possibility to affect the entire food chain.

In developing countries, additional problems arise such as the lack of know-how of their proper use, non-availability of suitable application equipment, inadequate storage conditions and high prices. The above problems have necessitated a revision and a new approach to pest control strategy and practice.

One area of investigation is the search for natural control agents among plants, i.e. secondary metabolites that may have insect repelling, insecticidal, antihormonal or antifeeding characteristics.

Sudan, with its area of one million square miles, covering all the geographical zones from the desert to the evergreen, is one of the richest countries in its flora. Plants of such a country, both cultivated and wild, undoubtedly represent an unlimited source for pharmaceutical and agricultural active agents to be used in pest control measures.

In this regard, the important research work with various plant species for their anti-feeding effects undertaken against certain insect species which are pests on crops, is reported here. In addition, the future considerations for the use of the most potential plant products is also indicated.

NEEM AND ITS PRODUCTS

One of the most important plants known to possess insect control potential is neem, *Azadirachta indica* A. Juss. The revival of neem's traditionally well known repellent and insecticidal properties stimulated a world-wide interest in this plant.

It was introduced to Sudan and the rest of Africa from South-east Asia at the beginning of this century as an ornamental avenue tree. It prevails everywhere in Sudan, because of its rapid growth, remarkable adaptability of withstanding vast climatic and geographical variations, and resistance to drought.

Chemical investigations on neem began in the 1950s. Mitra (1963) reviewed the chemistry of the active components of neem. The anti-feedant and growth-inhibiting effects of neem derivatives against a wide range of insect pests have been reported (Warthen, 1979; Saxena, 1981).

Recently, Broughton *et al.* (1986) reviewed the structure of neem principals related to three insect feeding inhibitors, viz., meliantriol, azadirachtin and salannin, which belong to a group of ranortepenoids. In addition to the anti-feedant action, azadirachtin and neem seed derivative have other pronounced behavioural and physiological effects (Schmutterer *et al.*, 1981; Schoonhoven, 1982; Saxena, 1983; Schmutterer and Ascher, 1984).

Neem research considerably proceeded and was highlighted in the First, Second and Third International Neem Conferences held in 1980, 1983 and 1986, respectively. The proceedings of these events provide a comprehensive survey of neem's broad insecticidal properties.

Stored Grain Pest Management

Considerable work has been done on protecting seeds and grains from major storage pests i.e. Bruchids, Khapra beetle, *Tribolium* etc. by using different neem products (Jotwani and Sircar, 1965; Deshpande, 1967; Jilani and Malik, 1973; Golob and Webley, 1980; Jilani and Su, 1983).

In Sudan, Sidding (1981) found that neem seed powder was effective and long lasting for the treatment of stored wheat against *Trogoderma granarium*. Abu Nayib (1989) reported that in all treatments with neem seed powder (0.5–2.0 g seed powder/50 g sorghum seeds) and the higher concentrations of neem leaf powder (2.0–4.0 g leaf powder/50 g

sorghum seeds), no adult beetles were observed and most of the larvae were dead. The few living larvae were undersized compared to the larvae in the control treatment, which could be considered as an indication of probable starvation caused by the antifeeding effects of neem components. The observed adults' malformation and death of some pupae could be accounted for by the probable growth regulatory effect of neem components reported by other workers (Ruscoe, 1972; Steets, 1975; Redfern *et al.*, 1981). The results also showed that neem seed powder was clearly more effective than either the neem leaf powder or the neem bark powder.

The methanolic neem seed kernel extract gave good control against the red flour beetle, *Tribolium castaneum* and concentrations of 1–10%, where the mortality of adult beetles reached 15% in the higher concentration. The development of the beetles was also significantly affected as the number of larvae produced decreased steadily with the increase in the methanolic neem seed kernel extract between 0.01 and 10% concentrations, reaching zero at 10% concentration (Abu Nayib, 1989).

Integrated Pest Management for Potato Insect Pests

Potato is gaining increasing importance as a vegetable crop in Sudan. To avoid the application of chemical insecticides on such a tuber crop, which could lead to the accumulation of pesticide residues in the tubers, an integrated pest management approach was adopted at Shambat Research Station to combat the tuber worm, *Phthorimaea operculella* and the foliage insect pests such as: Aphids, *Aphis gossypii*, leaf hoppers, *Empoasca lybica*, the whitefly, *Bemisia tabaci* and the cutworm, *Agrotis ipsylon*.

Previous results collected during the first stage of the programme (Sidding, 1986) showed efficient control of the tuber worm by cultural practices such as earlier sowing data, more sowing depth and early harvesting, while foliage insect pests were efficiently controlled by spraying with neem seed aqueous solution.

In the second stage of the programme, the research-managed trial, a package of recommended treatments was compared to a package of ordinary methods of planting potato by the farmer. The former, which included early sowing, sowing tubers at 3 inches depth, early harvesting and spraying with neem, showed significantly lighter infestation with pests and out yielded the latter package by one ton/acre in the first season and 2 tons/acre in the second season.

In the third stage of the programme, the farmer-managed trial, the following recommendations were drawn out:

1. Splashing as a method of applying neem followed by spraying, resulted in the best significant control of potato pests and accordingly in significant increase in yield.

2. Neem seed water extract at the rate of 1 kg powdered seeds/40 litres of water was recommended to small-hold farmers of vegetables by splashing, using twigs of neem branches.

3. In the case of larger areas of vegetables, neem seed water extract at the same rate to be applied using a knapsack sprayer.

Other Crop Pests

In the Sudan, the Gezira is the main agricultural area, comprising about two million acres. Intensive annual application of large amounts of pesticides against insect and weed pests of cotton, wheat, groundnuts and other crops is practised. The misuse of these pesticides created many problems and as a result of acquiring resistance to pesticides, some minor insect pests became major pests. One such insect is the cotton whitefly, *Bemisia tabaci*, which is considered now as the number one pest of cotton and other crops in Sudan. This has necessitated a revision and a new approach to pest control strategies and practices. One area of investigation is the search for natural control agents among plants.

In field trials involving neem seed and neem leaf aqueous extracts, spraying of cotton with concentrations equivalent to 1 kg of neem powder/20 litres of water and 1 kg/40 litres significantly reduced whitefly population two days after spraying. The higher concentration of aqueous neem seed extract gave even better results when compared to the application of an insecticide mixture of Mitac 20% E.C. +Endosulfan 50% E.C. at a dose of 1.4 1/acre (Abu Nayib, 1989).

Neem derivatives were also effective against the African melon ladybird beetle, *Henosepilachna elatrii*. Siragelnour (1986) reported that the development of immature stages was adversely affected by the neem aqueous extract. The affected larvae abandoned the treated leaves before the commencement of pupation and failed to develop into adults. Emerged adults had poorly developed and twisted elytra, while some adults only partially emerged from the pupal cases. The total egg production, number of egg batches, oviposition period and adult

longevity were all affected by feeding the adults on neem-treated leaves.

POTENT PLANT SPECIES

Sudan, being a large tropical country, provides ample plant sources free from pest infestation, which encourage taking up research work on many plant species for their possible use in pest control measures.

Rehan, *Ocimum* spp.

The genus *Ocimum* of the Labiatae family includes between 50 and 60 species of low growing shrubby plants, all more or less strongly scented due to the presence of essential oils. They are found distributed throughout the Old World tropics, from central Africa through Asia to parts of Australia (Cobley, 1963). Six species of *Ocimum* were reported to occur in Sudan by Andrews (1958). *Ocimum* species are annual or perennial and little is known in cultivation except for basil, *Ocimum basilicum* L., which is cultivated in some parts of the tropics for its essential oil, used for scenting of soap, in perfume industry and flavouring of medicines.

Guenther (1961) reported detailed information on the physiological properties of the essential oils of eleven species of *Ocimum*. Hochammer *et al.* (1946) studied two kinds of rehan oil produced in Egypt under the so-called white and red basil oil and found linalool and other component in both types of oil. In addition, the white type contained methyl cinnamate and the red type contained methyl chavicol and traces of cinnamic acid ester.

Insecticidal activities: The insecticidal properties of rehan, as reported by several workers, included the repelling of mosquitoes in West Africa by burning, protection of yams against termites and control of field crops and stored grain insects (Wat and Breyer, 1962; Molaka, 1972; Deshpande and Tipins, 1977; Rajendran and Gopalan, 1979).

In Sudan, preliminary investigations carried out by Gubara (1983) revealed that sorghum seeds treated with ground leaves of *O. basilicum* gave the least damaged sorghum grains by *Trogoderma granarium*. The damage was 13.7% compared to 87.3% in the control after 6 months of storage.

Hassan (1988) showed that rehan essential oil showed a typical insecticidal activity against the red flour beetle, *Tribolium castaneum*. The

oil was effective against the mosquito, *Culex* spp. and was found to be anti-feedant against the African melon ladybird beetle, *Henoseplichna elatrii* and the migratory locust, *Locusta migratoria*.

Abu Nayib (1989) reported that rehan leaf powder has the potential to give considerable protection to stored grains for a period up to three months against the damage by the Khapra beetle. The inflorescence powder contained lower concentrations of the active ingredients compared to the leaves.

Rehan leaf aqueous extract seems to have no effect in reducing whitefly or jassid populations on cotton in the field, which may suggest that the active agents are insoluble or sparingly soluble in water. On the other hand, rehan extracted by steam distillation gave significantly lower whitefly populations in counts made 2 and 6 days after spraying, when compared with control treatments.

Anti-fungal activities: The essential oils from *Ocimum* spp. and their components were reported to show different inhibition effects against several fungi (Afifi, 1975; Kurucz and Hornok, 1979; Rewni *et al.*, 1984; Asthana *et al.*, 1986).

In Sudan, Osman, (1986) reported that all concentrations of *O. bassilicum* oil (i.e. 1, 10, 20, 30 and 40 ppm) has completely inhibited aflatoxin production by *A. flavus* and that fungal growth was significantly reduced with increase in oil concentration.

With regard to the fungitoxic properties of rehan essential oil, Hamid (1989) found the oil to be very effective in inhibiting the growth of *A. flavus* with MIC of 0.08%. The nature of toxicity was found to be fungicidal at its MIC. Moreover, the oil was found to be more efficacious than two of the conventional synthetic fungicides used, viz., carbendazim (Bavistin I) and Zineb (Diathane Z-78).

Volatile assay of rehan essential oil showed a high efficacy against *A. flavus*. It inhibited the fungus growth completely at 0.14%.

The fungitoxicity of rehan oil showed a long storage life. The activity of the oil was not affected for up to 360 days, thus meeting one of the important criteria of a fungicide i.e. being stable for a long period of storage.

Rehan essential oil showed a broad spectrum of activity against a number of fungi. Eight of nine fungi tested were completely inhibited by the different concentrations of the essential oil used.

With respect to phytotoxicity, rehan oil was found to be non-phytotoxic to seed germination of sorghum. The growth of roots and shoots were also not affected. The oil has no adverse effect on the morphology and health of sorghum seedlings.

Since peanut can be severely infected by *Aspergillus*, a study was conducted to investigate whether rehan oil would inhibit the fungal growth and toxin production on this substrate. It was found that addition of rehan essential oil to water soaked peanut samples resulted in decreased fungal infection and aflatoxin production (Hamid, 1989).

In addition to the insecticidal activity, local availability, ease of application and biodegradability, it appears possible that the oil of *Ocimum* spp. might be exploited as: (a) A preservative for groundnut seeds during storage and transit, especially in Western Sudan where higher risk of aflatoxin contamination exists; and (b) Post-harvest grain protectant, especially in closed system of storage (e.g. ground pits) and for the control of post-harvest diseases of fruits and vegetables.

Henna, *Lawsonia inermis* L.

Lawsonia inermis L., locally known as henna, is presently used mainly in Africa and India as a dyeing material for hair and staining of hands and feet. This is mainly due to the presence of lawsone (2-hydroxy 1,4 naphthoquinone), which was reported to have bacteriostatic action (Karawya *et al.*, 1969). It has also been reported that henna was used by ancient Egyptians for preservation of mummies. Henna belongs to the family Lythraceae and the genus *Lawsonia*, has three species, viz., *L. inermis*, *L. alba* and *L. spinosus* (Dalziel, 1937), of which *inermis* and *alba* are found in Sudan (Andrews, 1958).

Henna was originally introduced to Sudan and used as a minor crop grown as a quick fragrant hedge, propagated by cutting. It is now cultivated in the Northern Region in an area of about 6000–7000 acres.

In order to investigate the possible utility of henna in pest control measures, Sanad (1987) tested the anti-feedant and insecticidal properties of plant material collected from different localities in Sudan.

When ground henna leaves were used as seed dressing, they showed an anti-feedant effect and the damage in seeds was reduced to 48% compared to 87% damage in the control. Aqueous henna leaf extract had no effect on the damage of seeds by insects pests or adult emergence of the insects. On the other hand, alcohol extract of henna leaf gave good protection to sorghum seeds, where only 1% damaged seeds were observed, while the control recorded 22% damaged seeds.

The chloroform of extract henna leaf was very effective and only 5% adult emergence occurred, compared to 22% in the control. When first instar larvae of the spiney bollworm, *Earias insulana* were fed on unripe okra seeds sprayed with 1% chloroform extracted from henna leaves, the growth and development of the larvae were retarded, pupal duration was lengthened and the longevity of the adults was significantly reduced.

The results of these trials indicated an insecticidal as well as an anti-feedant effect of the organic extracts of henna. The degree of inhibition depended upon the insect species, the henna source and the extractant used. In this respect, chloroform and ethyl alcohol were the solvents preferable in extracting lawsone from henna leaves.

Balanites, *aegyptiaca* Del.

The soapberry tree, *Balanites aegyptica* locally known as heglig, is widespread throughout the greater part of Sudan, right up to the Northern Frontier. The timber is used for making agricultural implements, local furniture and provides good firewood and charcoal. The fruits (known locally as laleb) are edible, and the inner core of the seed is crushed to provide 48% of a golden yellow oil suitable for soap making and cooking. The fruit and oil are used for medicinal purposes. Lately, the oil was reported as a molluscicide used to control snails in irrigation canals against bilharzia in Egypt.

Preliminary investigations were carried out at the Department of Crop Protection to detect any possible insecticidal, or anti-feedant activity of the oil. Laboratory reared 1–2 day old adults of cowpea bruchid, *Callosobruchus chinensis* (L.) were used in this study. Preliminary results indicated an insecticidal, anti-hormonal and anti-feedant characteristic of the oil from the soapberry tree. These results justify the need for further research on the utilization of the oil and more purification and extraction of the individual components might probably reveal some active and specific control agents.

FUTURE CONSIDERATIONS AND CONCLUSION

Plant derivatives possessing insect repellent and anti-feedant properties offer a novel approach in the management of crop and stored grain insect pests. Although the existence of botanical pesticides dates back a long time, the efforts to use these natural pesticides sources as alternatives or complements of synthetic pesticides is still limited. Plants such as neem, rehan, henna and heglig, which are rich sources of anti-feedant materials, are widespread in many developing countries and have shown promising results against a vast number of insect pests. These results justify the need for further research on the utilization of these plants and more emphasis should be given to further improvement by suitable amendments with additives, synergists and other botanicals, and by innovative formulations and methods of application. This will pave the way for an alternative pest management with less likelihood to disrupting the ecological balance.

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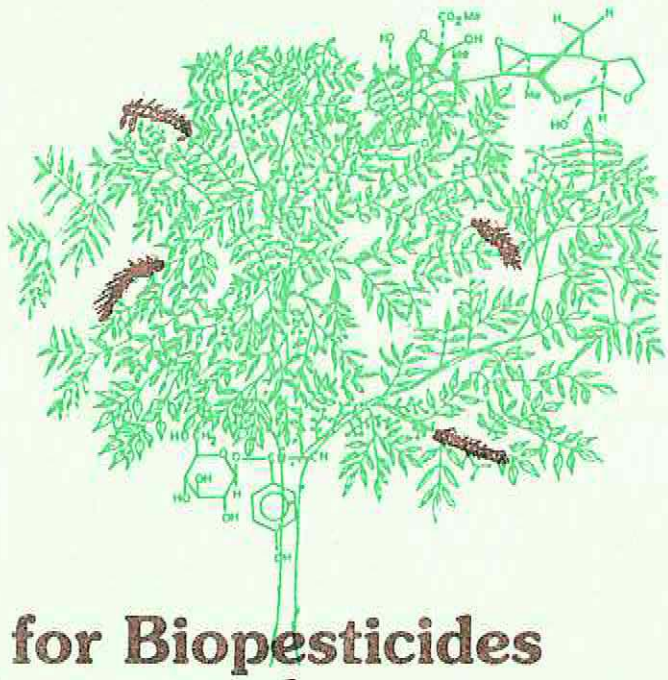

Scope for Biopesticides in an Integrated Pest Management Strategy for Mauritius

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Scope for Biopesticides in an Integrated Pest Management Strategy for Mauritius

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INTRODUCTION

Mauritius has traditionally been an agricultural island, ever striving, and successfully so, towards attaining greater food self sufficiency. Sugarcane is the backbone of the economy, as the major cash crop after tea. In this endeavour, pest control has been and still is one of the day-to-day agricultural pre-occupations to ensure food crop production, both quantity and quality wise with the limited agricultural land available (10,000 ha, or 10% of total agricultural land). Not surprisingly then, industrial chemical pesticides constitute a major input (besides chemical fertilizers) into the agroecosystem that could endanger human health, wildlife and the environment generally, and induce pesticide resistance buildup.

Concern about excessive pesticide usage in the food crop sector has already been raised elsewhere (Fagoonee, 1984a,b; 1978). The sugarcane crop pests are fortunately spared of insecticidal control, but this crop absorbs the bulk (95%) of the herbicides imported. The agricultural authorities, research institutions and consumers alike are very much conscious of the hazards of excessive pesticides use. Surveys are carried out from time to time to monitor pesticide practice and pest and pesticide perception amongst the farming community (Fagoonee, 1984b; 1987). Extension agents are active on this front, giving advice on the rational use of pesticides. However, the prevailing situation is yet far from satisfactory although improvements are being recorded (Fagoonee, 1987).

It is, therefore, the policy to develop alternative pest management methods and systems which would reduce dependency on chemical pesticides, efficacious, environmentally safe and cost-effective. An integrated pest control strategy seems to be the ideal solution, provided that a systems approach is adopted, based on hard baseline information on the ecological (biotic and abiotic) and other characteristics of the

major pests, not excluding some economic interface considerations. In addition, basic research and development research on alternative control methods should prove promising in the field, as gauged by standardized field experimentations and data collection. Viable options that offer promise include the use of naturally occurring plant allelochemicals, *Bacillus thuringiensis* and or other *Bacillus* spp. and strains, viral pathogens, pheromones and insect parasites. The current pest control problems already augur for a gradual shift in pest management strategy from chemical and biological controls to an integrated approach.

CURRENT PEST CONTROL EFFORTS

Chemical Control

Over the years, much information has been gathered, perhaps less disseminated, on the major pests of major crops as well as livestock. The main thrust in the control, is through the application of chemical pesticides. It is obvious that a wide range of chemicals is in use, ranging from organophosphates, carbamates to pyrethroids; organochlorines are fortunately being phased out. Over a dozen products are applied just on the solanaceous crops, potatoes and tomatoes. The introduction of pyrethroid products such as deltamethrin (Decis) and cypermethrin (Cymbush) has, however, caused a shift in their favour (Fagoonee, 1984b; 1987).

The Entomology and Plant Pathology Divisions of the Ministry of Agriculture and the Mauritius Sugar Industry Research Institute make recommendations normally through the Extension Division of that agency regarding proper pesticide utilization and handling, dosage, application frequency and time, recommended safety intervals, use of pesticide mixtures, sprayer characteristics, protective clothing and disposal of empty containers. Awareness campaigns on the adverse effects of excessive and irrational pesticide use are regular feature activities by way of the media, information booklets, farming news bulletins, posters, talks and seminars.

Biological Control

In the field of biological control, Mauritius has had several successes as well as failures. The successful introduction in 1762 of the mynah bird *Acridotheres tristis* from India to keep down the red locust *Nomadacris septemfasciata* population breeding on sugar cane, has been a landmark in biocontrol not only for Mauritius but for humanity at large. The other

successful biological control agents concern the parasites introduced from elsewhere for sugarcane pests. Thus, the population of *Clemora smithi*, *Sesamia calamistis* and *Chilo sacchariphagus* are till today kept below economic thresholds. All their parasites have become well-established, thus saving the cane planting community from having recourse to expensive chemical pesticides. Extreme caution is, however, exerted as: (a) we get involved in cane interline cropping food crops which necessitate pesticide applications (potatoes, tomatoes, haricot beans), and (b) we modify the cultural and harvest practices of the sugarcane crop via mechanization and preharvest field burning. Close monitoring is warranted to watch for the critical point in the time when that stable ecosystem gets upset; the possibility then of pest resurgence cannot be ruled out.

In so far as crop pests are concerned, entomophagous species recorded for *Liriomyza trifolii* are: Eulophidae, *Hermiptarsenus semialbiclava* Girault (commonest), *Meruana* spp., *Chrysonotomyia* spp., Cynipoidea and an unidentified cynipid (Fagoonee and Toory, 1984). These would control 50–70% of the natural leafminer populations; such a check in the population buildup is not sufficient, being given the high biotic potential of the pest.

Parasitic control is also recorded on citrus: the whitefly *Aleurothrixus floccosus* is checked by *Cales noacki*; the psyllid *Trioza erytreae* is controlled by *Tetrastichus dryi* whereas another psyllid *Diaphorina citri* is checked by *Tetrastichus radiatus* (Joomaye, 1988).

Significant biological control of stableflies *Stomoxys nigra* and *S. calcitrans*, which bite cattle and deer, is achieved by *Tachinaephagus stomaxicida* (Encyrtid) together with *Trichopria* sp. (Diapriidae) and *Spalangia* sp. (Pteromalidae) (Fagoonee, 1984a).

A major breakthrough was achieved by the successful introduction of *Rhabdionvirus oryctes* from Malaysia in 1970 to control the devastating coconut beetle *Oryctes rhinoceros* (Monty, 1978).

Attempts to introduce parasites for fruitfly control have not proved successful (Fagoonee, 1984a).

Pheromones

Not much has been done by way of pheromone research in Mauritius. The author used Trimedlure to monitor the fruitfly *Paradalaspis cyanesces* population buildup in tomato *Solanum esculentum* cultivations

(Fagoonee, 1977). The Ministry of Agriculture monitored the population dynamics of the fruitfly release programme (1977 to 1981) by using the pheromone Trimedlure in baited flytraps (Fagoonee, 1984a).

The Mauritius Sugar Industry Research Institute started pheromone research for monitoring the population dynamics of *Chilo sacchariphagus*.

Biopesticides

For more than a decade, the School of Agriculture of the University of Mauritius has been devoting sustained effort in developing biologically active substances from local plants to control pests. Laboratory and field trials have, for example, confirmed the efficacity of controlling cruciferous pests *Crocidolomia binotalis* (cabbage webworm), *Plutella xylostella* (diamondback moth) and the serpentine leafminer *Liriomyza trifolii* by extracts and the pure bioactive compound azadirachtin from the neem or Persian Lilac plant *Azadirachta indica* A. Juss. (Meliaceae) (Fagoonee, 1980, 1981; Fagoonee and Lauge, 1981; Fagoonee and Toory, 1984). The effects of neem extracts and azadirachtin are polyvalent: anti-feeding growth regulating through disruption of the endocrine system, inhibiting oviposition, and repellant to moths. In addition to action by contact and ingestion (stomach poison), neem is also systemic, capable of being absorbed through roots into the foliar system. In field applications, cabbage and onion plantations have been successfully controlled using aqueous neem extracts at doses ranging from 0.5% for *L. trifolii* (on onion leaves) to 2% on crucifers.

Another plant well studied is the goatweed *Ageratum conyzoides* L. (Compositae); (Fagoonee and Umrit, 1981) whose bioactive substances are precocenes 1 and 2, the same chromenes identified by Bowers *et al.* (1976) in *A. houstonianum* in the bedding plant. Crude lipid extracts exert ovicide action and induce sterility after topical treatment of both young last instar larvae (L_6) and females of *Dysdercus flavidus* Sign. at emergence, by inhibiting ovarian development. The effect lasted for at least 12 days, the two precocenes I and II being synergistic.

Currently, several investigations are in progress (S. Facknath, pers. comm.):

- the effect of *Artemisia absinthium* on *Crocidolomia binotalis*; a 10% ethanolic extract causing upto 68% mortality;
- the repellent effects of 4% ethanolic *Argemone mexicana* extracts on *C. binotalis* moths and larvae;
- the anti-feedant and IGR (insect growth regulating) effects of

extracts from *Siegesbeckia orientalis, Cassia ocidentalis* and *Citronella* on *C. binotalis* on *Dysdercus flavidus*. *Citronella* is also exhibiting repellency.

Preliminary results are already promising. With a flora of over 2000 local species with medical and other biological properties, a systematic screening programme is envisaged.

FUTURE PEST MANAGEMENT PROGRAMME

With the need for environmental protection and flora and fauna conservation, our research and development in pest control programmes should aim at slowly decreasing the use of toxic chemical pesticides. Such a strategy could in the immediate future phase out a certain number of chemical products having similar biocidal effects or same target species, whilst taking into consideration the impending cross- and multiple-resistance building implications. Much pure and applied research has been carried out on alternative pest control methods in research institutes elsewhere, and it is opportune that advantage is taken of the same. The ultimate aim is to re-establish an ecologically sound agroecosystem. Consequently, in the short term, the objective would minimize pesticide input into the agroecosystem and, in the medium to longer term, implement alternative pest management methods in an integrated way.

Minimizing Pesticide Utilization

In view of a better and rational pesticide management, a masterplan for pesticide use has become expedient. The fate of pesticides in the ecosystems—terrestrial and aquatic — has not been qualitatively nor quantitatively studied. The masterplan would examine in depth existing legal and institutional frameworks in order to come up with a consolidated foodcrops, milk and organisms and functional strategy. Pesticide residues have to be more closely monitored. In this connection, laboratory facilities have to be strengthened. At the same time, a pesticide array laboratory has to be put up in order to carry out quality control on imported pesticides. At the level of pesticide sellers and users, a multi-disciplinary team should carry out surveys on perceptions of pests and pesticides, examine the effectiveness of existing educational and extension programmes, and develop communication materials for improving such activities. Presently, it is recommended that ULV (Ultra Low Volume) and electrostatic sprayers be introduced in order to save the quantity of chemical products used as well as to prevent runoff and unnecessary loss through drenching.

Use of Botanicals

With the availability of viable and stable commerical preparations of neem extracts, it is urgent that, in the short term, these 'products' be tested on pilot field trials on state-owned demonstration farm centres before recommendations are to be passed on to the planting community. Being naturally occurring biological products, these will become popular very fast.

At the same time, a neem tree plantation campaign will have to be launched. Since pure stand plantations do not come up well and land is scarce, it is proposedto grow them along roadsides and around cave and other fields as well as hill slopes. This would eventually facilitate seed collection.

Meanwhile, developmental research should continue towards locally produced stable formulations of neem extracts from seeds produced from existing neem trees. The use of stabilizers, adjuvants antioxidants, UV protectants and synergists should be recognized. Other potential botanicals have to be looked for from existing rich indigenous flora. Botanicals have prospects for both crop pests as well as stored product pests.

Microbial Control

Bacillus thuringiensis has been commercialized for more than two decades, but has had limited success in the control of *Heliothis armigera.* However, with the advances in *B. thuringiensis israeliensis (Bti)* research through genetic engineering, fast screening and production of strains, it is now possible, in a relatively short time period, to isolate local *B. thuringiensis* strains and mass produce them in fermentors; the latter technology has been well mastered now and emphasis is laid on the use of local raw materials (Dharmsthiti *et al.*, 1985) as culture media (yeast hydrolysates, carbohydrate source) and for formulations (coconut husks or wheat bran). Collaborative effort with laboratories abroad is essential. *B. thuringiensis* is safe to the environment, does not maintain itself in the ecosystem, and does not adversely affect aquatic fauna. Another area where interest should be focused is the development of viral pathogens, especially baculoviruses which are known to be stable and specific.

Biological Agents

Despite the excessive use of pesticides in the agroecosystem, some pest

parasites are often obtained in laboratory breeding. It is, therefore, necessary to carry out an inventory of existing parasites, breed them, evaluate their effectiveness and monitor their eventual mass release in the field. Concurrently, exotic species have to be introduced, bred and assessed.

Pheromones

A strong pheromone research programme has to be developed. Such a programme will focus initially on fruitflies (in both food crops and fruit trees). Whilst the use of baited traps is to be envisaged, pheromones should be integrated with other control methods in order to enable concomitant monitoring of the pests population dynamics and, hence, the performance of control measures.

In the field of livestock pests, the strategy developed at the International Centre of Insect Physiology and Ecology (ICIPE) in Nairobi for the behavioural control of tsetsefly is worth adapting to the stableflies *Stomoxys nigra* and *S. calcitrans*. The project consists of identifying long range, medium to short range, as well as proximal cues that determine host selection of the pest. The next phase would consist of modifying these factors and causing total 'confirm' to the pests.

International Collaboration

For any integrated pest management (IPM) programme to succeed, a systems approach is recommended. The individual components of the IPM for each pest and their interactions have to be well defined, for eventual integration. Only when all the biotic, abiotic and social interface parameters have been established, can a model be worked out, tried and modified if need be. It is, therefore, essential that a local scientific team closely works out the programme, plan the work, implement it and regularly monitor performance. It is equally paramount that international collaborative agreements be sought with developed centres of excellence for exchange of scientists, training and exchange of information. The ARPPIS and PESTNET programmes developed and based at ICIPE fit appositely in this context. Financial support from donors is pledged.

In pursuance of such collaboration and information exchange, it is proposed that a *B. thuringiensis* R&D Newsletter and a Pheromone R & D Newsletter be launched along lines of the existing Neem Newsletter distributed by the Indian Agricultural Research Institute (I.A.R.I.). In addition, research grants should also provide for: (a) subscription to

scientific journals and membership to scientific professional societies, (b) acquisition of backup computers, and (c) participation in regional and international workshops, seminars and conferences.

Biotechnology

Developing countries and other centres of excellence having the human, technological and financial resources should be encouraged to venture into development of genetically engineered systems for the production of semiochemicals and pheromones from, for example, cell cultures or induced plant systems. Genetically altered bacteria, baculoviruses and fungal pathogens are other possibilities. Microbial production of certain behavioural lures (e.g. phenolic compounds) in slow release systems offers promise in livestock and crop pest management. Biotechnological applications in agricultural, veterinary and medical vector pest control are virtually unlimited (Anon, 1987, 1990).

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Biological Control of *Sclerotium rolfsii* in Tomato and Peanuts

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Biological Control of *Sclerotium rolfsii* in Tomato and Peanuts

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INTRODUCTION

The potential of the genus *Trichoderma* as a biopesticide was first recognized by Weindling (1937) who isolated fungitoxic substances from culture filtrates of *Trichoderma*. Since this initial work, the ability of this fungus to control other fungi has been amply documented (Dennis and Webster, 1971a, b.; Elad *et al.*, 1980; Elad *et al.* ,1982; Papavizas, 1985). Among the soil borne pathogens which have been controlled with much success using *Trichoderma* spp. are *Fusarium* spp., *Pythium* spp., *Phytophothora* spp. and *Rhizoctonia* spp. The mode of action of *Trichoderma* is mainly parasitic and B 1,3 glucanase and chitinase secreted by the mycoparasite as constitutive enzymes are involved in the degradation of the pathogens (Hoch and Fuller, 1977; Elad *et al.*, 1983a,b).

Sclerotium rolfsii on the other hand is a facultative parasite of the root collar and roots of over 200 plants (Aycock, 1966). It is of economic importance on various legumes and other cultivated plants including tomatoes and pepper (Domsch *et al.*, 1980). In peanuts, for example the disease caused by *S. rolfsii* may destroy the plants completely, leading to a soft rot of the pods and substantial loss of yield (Rodriguez-Kabana *et al.*, 1975).

In Ghana, though data on yield loss due to *S. rolfsii* attack of tomatoes and peanuts are scanty, observations have shown the disease to constitute a great menace to the two crops as well as many other vegetable and field crops. Its impact on forest crops is not yet documented.

This paper reports the distribution of *Trichoderma* spp. in some Ghanaian soils, the preparation of *Trichoderma* inoculum on local agricultural and forest wastes, and the use of such *Trichoderma* inoculum in the control of *S. rolfsii* in some agricultural crops.

DISTRIBUTION OF *TRICHODERMA* SPP. IN SOME GHANAIAN SOILS

In an earlier study of the incidence of damping-off in tomatoes grown in various soils, it was detected that the disease was less common on tomatoes grown in virgin soils especially those with a forest cover. The explanation for this was the low population of the pathogen or the high population of the pathogen with a high population of the antagonist in the soil.

To test the second part of the hypothesis a quantitative estimation of *Trichoderma* species and its distribution in four soils with various vegetation cover and at different levels of cultivation — grass covered soil, forest covered soil, garden soil and a continuously cropped soil were studied. The count of *Trichoderma* populations in the soils was carried out on a *Trichoderma* selective medium (T.S.M) developed by Elad *et al.* (1981) using the serial dilution method in a plate count.

The result of the study showed that highest populations of *Trichoderma* spp. were found in the forest soil where little cultivation has taken place, whilst the lowest population count came from the soil which had been continuously cultivated with field crops and vegetables (Table 1). The garden soil and the grass covered soil were intermediate between the two and had similar population counts.

Table 1. Populations of *Trichoderma* spp. in different soil dilutions. Populations of *Trichoderma* in soils

Dilutions	Forest	Grass	Garden	Continuously cultivated
10^{-1}	*	*	*	16
10^{-2}	112	93	83	11
10^{-3}	100	65	65	9
10^{-4}	99	57	57	6

* Colonies too numerous to be counted.

This finding confirms that of Chet (1987) who found that soil taken from agricultural regions had low natural populations of *Trichoderma* spp. usually not exceeding 10^2 CFU/g.

All the isolates were *Trichoderma hamatum* which according to Rifai (1969) was the most common and widespread species in his study of the classification of *Trichoderma* using taxonomic, physiological and

biochemical markers. The ecological implication of this study is that *Trichoderma* populations decrease with increasing cultivation and depletion of the natural vegetation.

OBJECTIVES

The objectives of the study were:

(a) to control *S. rolfsii* attack in tomato and peanuts;
(b) to determine the best mode of application of the biocontrol agent;
(c) to determine the rate of development of the disease, and
(d) to determine whether *T. harzianum* could control *S. rolfsii* in peanuts and tomatoes in the field; and to assess the efficacy of the treatment.

MATERIALS AND METHODS

Production of *Trichoderma* Inoculum on Agricultural and Forest Wastes

Trichoderma inoculum for soil treatment is usually prepared on peat medium. To make use of the fungus as cheaply as possible in a country where this ingredient is to be imported, studies were carried out to find local substitutes for it, in order to constitute an effective but less expensive medium for the preparation of inoculum for field trials. The components used were sawdust, maize-bran, wheat-bran and rice-bran in combinations as shown in Table 2.

Sawdust mixed with maize-bran in different combinations proved to be the most suitable substrate for cultivating *Trichoderma*. A little addition of maize-bran to sawdust greatly improved the growth of the

Table 2. Development of *T. hamatum* on combinations of wawa sawdust and cereal brans

Substrate	1:0	1:0.25	1:0.50	1:0.75	1:1	0:0
	Radial growth (mm)					
Sawdust +maize-bran	0	85	87.50	88.50	90.00	88.50
Sawdust + wheat-bran	0	75	80.00	87.00	90.00	88.50
Sawdust + corn cobs	0	82	80.00	87.00	90.00	86.00
Sawdust + rice-bran	0	0	0	0	0	0

organism while the fungus was unable to grow on sawdust alone. Large quantities of wheat-bran were required to be added to sawdust before the fungus could grow well on the medium. The fungus could not grow on a combination of rice-bran and sawdust. Ground corn cobs and sawdust could also serve as a medium for the preparation of inoculum of *hamatum.*

Biocontrol of *S. rolfsii* in Tomato and Peanuts by *T. harzianum*

To determine the effectiveness of *Trichoderma* spp. as a biocontrol agent of tomato and peanut diseases caused by *S. rolfsii* in Ghana, an isolate of *T. harzianum* which has been shown or demonstrated to be effective in Israel against some strains of *S. rolfsii,* was used.

Green House Trials

Control of S. rolfsii *disease using direct soil inoculation* . Seeds of tomato variety Ayalon were sown in polyethylene pots filled with either sterilized or unsterilized sandy loam soil. One set of untreated soil was inoculated with a peat preparation of *T. harzianum* at 5 g per kilogram of soil. A second set of pots with sterilized soil was inoculated with *S. rolfsii* at 50 mg of sclerotia per kilogram of soil. A third set of sterilized soil was inoculated with both *T. harzianum* and *S. rolfsii* at the same dosage as the other two. Each treatment was replicated five times. The plants were observed for 30 days for seedling emergence. Diseased plants were rogued out at every inspection, counted and expressed as percentage infection.

Mode of application of biocontrol agent. In the second green house experiment the mode of application of *T. harzianum* as the biocontrol agent was studied by applying it as seed coating with conidial suspension. Direct inoculation to soil with a peat preparation in the following six treatments were performed:

1. Control
2. Inoculation with *S. rolfsii*
3. Direct inoculation with *T. harzianum*
4. Seeds coated with *T. harzianum*
5. Direct inoculation of *T. harzianum* and *S. rolfsii*
6. Seed coated with *T. harzianum* and soil inoculated with *S. rolfsii.*

A third green house experiment was conducted on tomato and peanuts to assess the ability of the biocontrol agent to control the incidence of the disease in tomato and peanuts.

The methodology adopted for experiments 2 and 3 were the same and is outlined as follows: Seeds were coated with 3 ml of conidial suspension containing 5×10^6/ml of *T. harzianum*. The seeds were immediately dried in a warm draft. Direct soil inoculation was done by applying 5 g of peat preparation to 1 kg of soil. *S. rolfsii* was usually inoculated at a concentration of 50 mg of sclerotia to 1 kg of soil.

The soil was sterilized by autoclaving for 2 hours at 121°C on two consecutive days. The treatments were replicated five times. The effectiveness of the treatments was assessed through (1) Seedling emergence; (2) Dry weight of plants; and (3) Reduction in diseased plants.

Reduction in disease was calculated using the formula derived by Hadar *et al.* (1979) and Elad *et al.* (1980) which states:

$$DR = \left[1 - \frac{(DC-DT)}{DC}\right] \times 100$$

where DR = % of disease reduction
DC = % level of disease in control plot
DT = % level of disease in treatments

Field Control of *S. rolfsii* in Tomato and Peanuts with *T. harzianum*

Two field plots each measuring 2.2 x 1.5 m were set up on a sandy loam soil which was known to have been heavily infested by *S. rolfsii* from previous studies. One seed of tomato was sown per hill at a planting distance of 30 x 15 cm. In all 40 seeds were sown to a plot.

The same plot size was set up for the peanut trials. However, two seeds were planted per hill at a planting distance of 40 x 20 cm. In all 40 seeds were sown on a plot.

The two treatments applied to the seeds were:

(a) seeds coated with a slurry of 3 ml *T. harzianum* conidia at a concentration of 5×10^6/ml and
(b) seeds dipped in water.

Each treatment was replicated four times. The seeds were immediately dried in a warm draft and sown.

RESULTS AND DISCUSSION

The results show that *T. harzianum* reduced the attack of tomato by *S. rolfsii* in the naturally infested and artificially inoculated soil from 80 to 37.5%, respectively and 95 to 27.5% (Fig. 1). These results are similar to those obtained by Chet *et al.* (1982) during the control of similar infections in Iris bulbs. At the beginning of the experiment, infection was lower in the naturally infested soil probably because of low inoculum potential of the pathogen. Garrett (1970) has noted a similar phenomenon. However, at the end of the experiment the level of

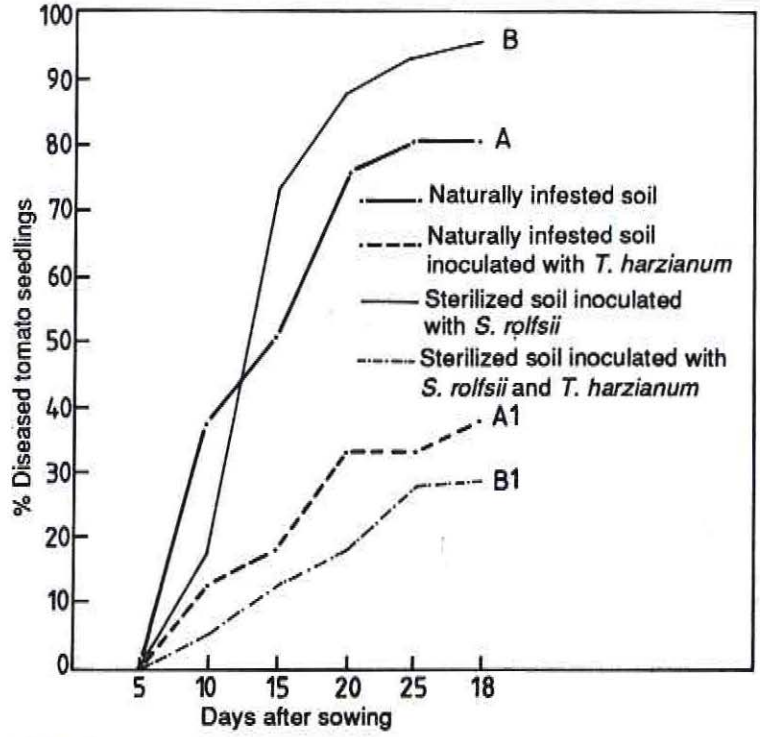

Figure 1. Development of root rot of tomato in treated and untreated soils inoculated with *T. harzianum* and *S. rolfsii*.

infection in the naturally infested soil was only 15% lower than the artificially inoculated soil, an indication of the high incidence of *S. rolfsii* in our cultivated soils.

The results on the mode of application of the biocontrol agent shown in Tables 3 and 4 indicate that a disease reduction as high as 70.6% was achieved for the tomato, while 52% was obtained for the peanuts when direct inoculation of the peat preparation of the *Trichoderma* was employed. Apart from the high percentage reduction

Table 3. Response of tomato to various applications of *Trichoderma harzianum* under green-house conditions

	Naturally infested			Artificially infested		
	Dry wt (g)	Emerg %	DR (%)	Dry wt (g)	Emerg %	DR (%)
Treatment						
Germination control	2.35	92	16	2.40	73	18
Disease control	1.32	92	–	1.30	65	–
Seeds coated with *T. harzianum*	2.48	92	56	2.31	82	43.60
Direct inoculation with *T. harzianum*	3.43	90	61	3.52	75	70.60

Table 4. Response of peanuts to various applications of *Trichoderma harzianum* under green-house conditions

	Naturally infested			Artificially infested		
	Dry wt (g)	Emerg %	DR (%)	Dry wt (g)	Emerg %	DR (%)
Treatment						
Germination control	4.50	90	59	5.70	90	34
Disease control	4.45	–	–	5.50	66	–
Seeds coated with *T. harzianum*	8.40	76	78	7.20	70	62
Direct inoculation with *T. harzianum*	10.50	78	15	12.00	80	52

of the disease in the case of the direct inoculation, crops subjected to this treatment had increased growth response and good crop stand as shown by the dry weight measurements.

Chet *et al.* (1987) have made similar observation which they explained that peat might be serving as carrier and food base for *Trichoderma*. They also suggested that *Trichoderma* might affect minor pathogens in the soil as well as the plants directly by excreting a regulating hormone which may in turn increase the growth rate or the efficiency of nutrient uptake.

Table 5. Reduction (%) of incidence of *S. rolfsii* in naturally infested soil as at the 15th day after germination in tomato

Treatment	Emergence at 6th day	Diseased plants at 15th day	% of diseased plants	% # level of diseased	Reduction % of disease
Control	4.1	1.8	42.0	38.1	
Seeds coated	4.6	0.9	19.0	14.7	22.7
Direct inoculation	4.3	0.7	12.0	10.5	28.0

Table 6. Reduction (%) of incidence of *S. rolfsii* in naturally infested soil as at the 15th day after germination of peanuts

Treatment	Emergence at 6th day	Diseased plants at 15th day	% of diseased plants	% Level of disease	Reduction % of disease
Control	7.0	4.0	57.7	45.7	
Seeds coated	8.5	1.5	15.6	14.7	37.9
Direct inoculation	8.4	1.2	13.0	11.4	41.2

Coating of the seeds with a conidial suspension of *T. harzianum* was more effective in controlling pre-emergence damping-off than inoculating the soil directly with a peat preparation of *T. harzianum* especially in the artificially inoculated soils. It decreased pre-emergence damping-off by 17 and 4% in the tomato and peanuts respectively.

Coating the seeds with the conidial suspension according to Chet (1987) endows the seeds with a protective device so that the infection courts are protected. This observation was also made by Rishbeth (1963) when he used *Peniophora gigantae* to control *Heterobasidim* (Fomes) *annosum*.

The level of disease in the field experiments (disease index) were 38.1 and 45.7 for the tomato and peanuts control, respectively (Tables 5 and 6). *T. harzianum* as peat preparation significantly reduced the level of disease by 28% while the conidial suspension reduced the level by 22.7% for tomato.

In the peanuts, direct inoculation by *T. harzianum* preparation reduced the level of disease by 41.2% while a disease reduction of 37.9% was obtained for the seed coating.

These results compare favourably with those obtained by Elad *et al.* (1980) where *T. harzianum* applied as wheat bran preparation reduced the percentage diseased plants by 20% and those by Sivan *et al.* (1984) where a disease reduction of up to 85% was obtained for tomato.

These studies have shown that inoculum of the biocontrol fungus *T. hamatum* can be prepared by cultivating it on inexpensive local materials that are non-foreign exchange dependent and found in all tropical countries.

CONCLUSION

The study has demonstrated the potential of *Trichoderma* spp. as a biological control agent for diseases of tomato and peanuts caused by *S. rolfsii* in a limited area of Ghana. However, a remarkable decrease in disease prevalence has not been produced. These studies are preliminary and further experimentation and improvement in the technique of application could expand the effectiveness of the control method.

It has also been established that *T. harzianum* is not indigenous to Ghanaian soils. In such introductions competition from local soil flora may limit the potency of the organism. It is, thus, intended to compare the effectiveness of *T. hamatum*, the indigenous *Trichoderma*, to *harzianum* in our subsequent experiments.

The limited scope of these experiments does not allow us to draw general conclusions on the applicability of this control measures on many of our soils in Ghana. It is intended to conduct trials in many parts of the country at a later stage.

Finally, the ecological significance of our observation on the effect of forest cover removal on populations of *Trichoderma* and *S. rolfsii* will be seriously pursued.

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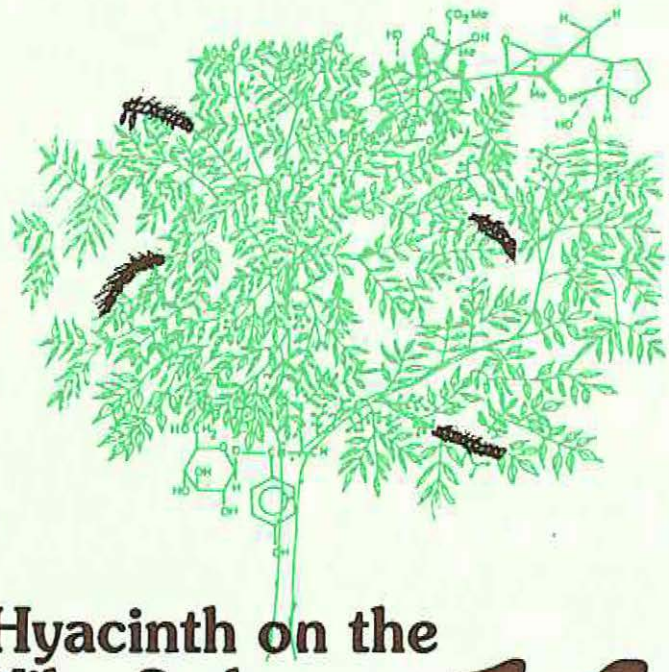

Water Hyacinth on the White Nile, Sudan before and after Biological Control

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M.O. Bashir

Water Hyacinth on the White Nile, Sudan before and after Biological Control

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INTRODUCTION

The water hyacinth *(Eichhornia crassipes* (Mart.) Solms-Laubach) was first seen in the Sudan in 1955/56 near Bor on Bahr El Jebel (Heinen and Achmed, 1964). Quite likely it got access to the Nile via the Zaire-Nile water shed (Heinen and Achmed, 1964; Mohamed, 1975). By 1960 the entire White Nile river from Juba to Jebel Aulia Dam 42 km south of Khartoum was infested. The plant was inaugurated as a national pest, considered as the third in such list after the weaver bird, and the desert locust and headed the list of the most damaging weeds in the country. In 1962 an organized control campaign was started, later assisted by the German GTZ technical and financial help where intensive use of the herbicide 2, 4-D was an eminent feature. The practice did not solve the problem and the least to say it was ecologically the most disruptive practice ever evidenced in that area.

Biological control of the weed was started through the help of the British ODA and CIBC in 1968. In 1979 with importation assistance from the USDA and NAS the two weevil species *Neochetina eichhorniae* and *N. bruchi* and the moth *Sameodes albiguttalis* were mass cultured and released against the weed. The three species were successfully established and their impact on the population and distribution of water hyacinth was evident.

This paper deals with the evaluation of the density, distribution and productivity of water hyacinth before and after the introduction and establishment of the three species of natural enemies for its control.

MATERIALS AND METHODS

Surveys to determine the population density and dynamics of water hyacinth in the navigable river section of the White Nile from Juba to Malakal were made twice annually. One during the rainy season (August–September) and the other during the dry season (January–February). During these surveys the absolute width of the two spatially distinct categories of the plant, (bank and floating) were determined

every five kilometres during navigation and particularly in the vicinity of outstanding landmarks such as side channels, lakes, lagoons and reference poles. Comparison of estimations made by different people was observed so as to determine the estimation error.

The reproductive rate of water hyacinth was also investigated under field conditions. The method used as early as 1967 consisted of labelling plants in the bank mats along the shore. Every 20 meters 25 plants in the middle of a stable mat were labelled. Each plant was disconnected from any daughter plants attached to it and labelled by a red tag carrying a number and date. The plants were inspected after 7, 14 and 21 days to record the number of daughter plants produced. The reproductive rate or increment factor and the doubling time were calculated using the method followed by Block (1969) and Freidel (1979).

The amount of water hyacinth that drifted and accumulated behind Jebel Aulia Dam every month during the rainy season since 1960 is recorded by the plant protection weed control section. Estimation of the area in acres was made so as to avail the needed control requirements. This was kept in record at the Jebel Aulia Dam Station. The data was compiled on annual basis and used to compare the accumulated hyacinth before and after the release and establishment of the natural enemies.

RESULTS AND DISCUSSION

The area infested by water hyacinth in the White Nile and its tributaries (Fig. 1) was estimated to be over 3200 km in length. The infestation zone extends far North of Bedden Rapids northwards up to Jebel Aulia Dam. Badden Rapids form a barrier, together with other hydrological features, to the expansion of the water hyacinth southwards. Jebel Aulia Dam forms an artificial barrier to its expansion northwards. Water hyacinth seemed to have reached the limits of its dispersal in the Sudanese White Nile system. Control of this aquatic weed was mainly through the use of the herbicide 2,4-D. This was administered through extensive aerial, river and ground campaigns (El Tigani, 1975). However, since 1983 little or no chemical control was applied due to security reasons.

Biological control of water hyacinth was attempted when the two weevil species *N. eichhorniae* and *N. bruchi* and the moth *S. albiguttalis* were imported and successfully established (Irving and Bashir, 1985; Bashir and Bennett, 1984).

Figure 1. Map of the White Nile system in southern Sudan infested by water hyacinth.

Studies on evaluation of the effect of these species on the productivity and density of water hyacinth were in effect since 1980. Results of surveys on the density and distribution of water hyacinth conducted before and after the release and establishment of the three natural enemies are presented in Figures 2 to 7. Figures 2 to 5 show the distribution and density of the floating and stationary bank hyacinth in the navigable stretch of the White Nile from north of Juba up to Malakal in the Southern Region of the Sudan. In this navigable lane it was quite easy to distinguish between the two categories of the plant as the lane is very narrow and it was easy to make reasonable estimation of the width of the area covered by the plant within a 10% level of error. It is evident that the area covered by both categories was small in 1984 compared to 1967. It is also evident that violent fluctuations in the population of the plant are markedly damped off, indicating stability particularly in the dry season (January–February) when disturbing physical factors such as wind, flood and rain are minimum.

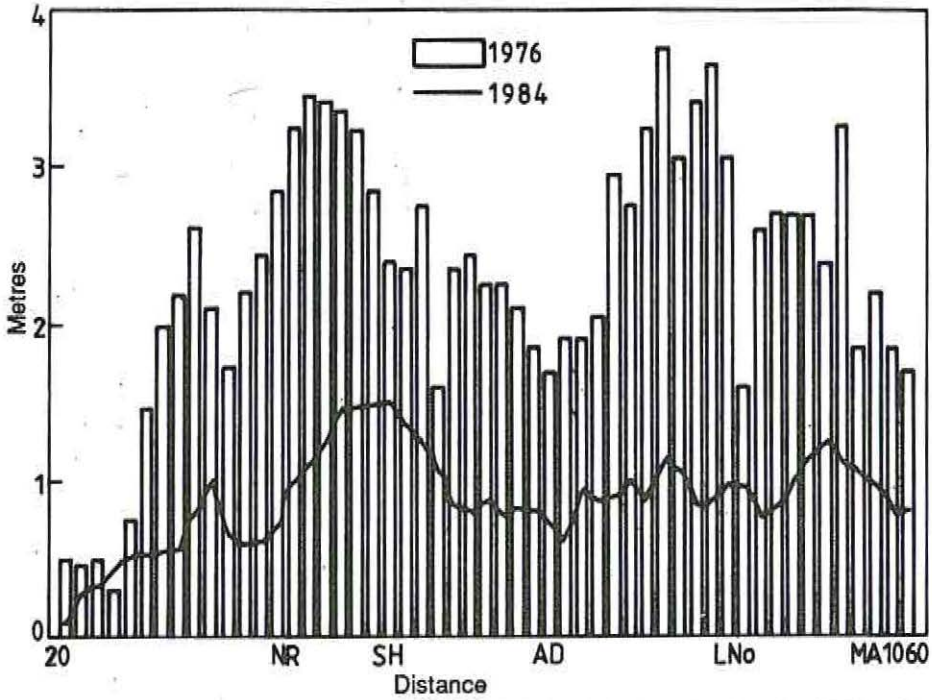

Figure 2. Density and distribution of bank hyacinth August–September 1976 and 1984.

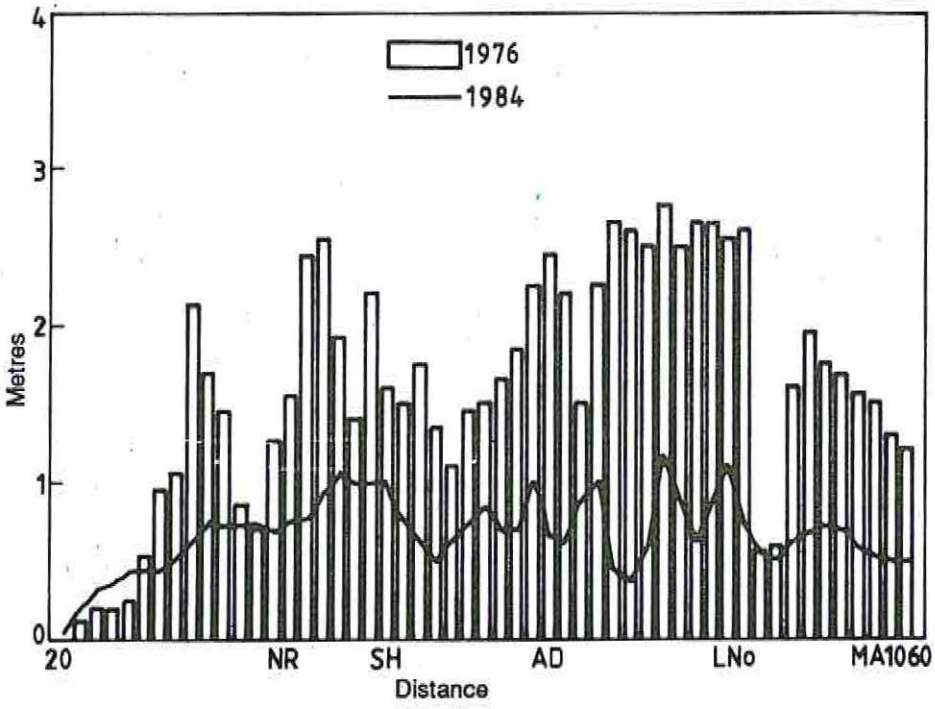

Figure 3. Density and distribution of floating hyacinth January–February 1976 and 1984.

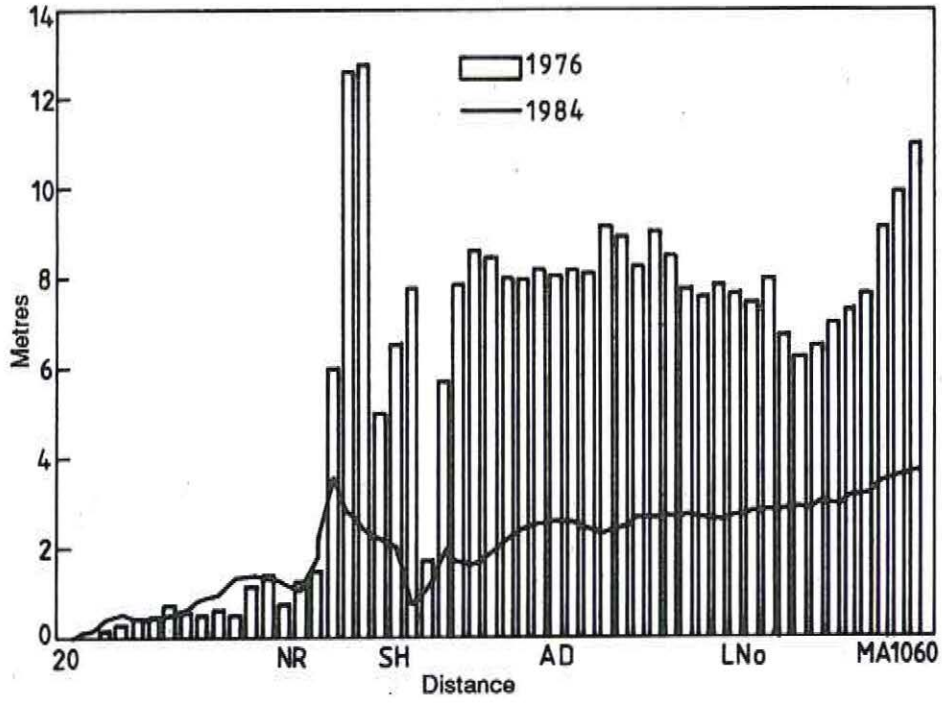

Figure 4. Density and distribution of floating hyacinth August–September 1976 and 1984.

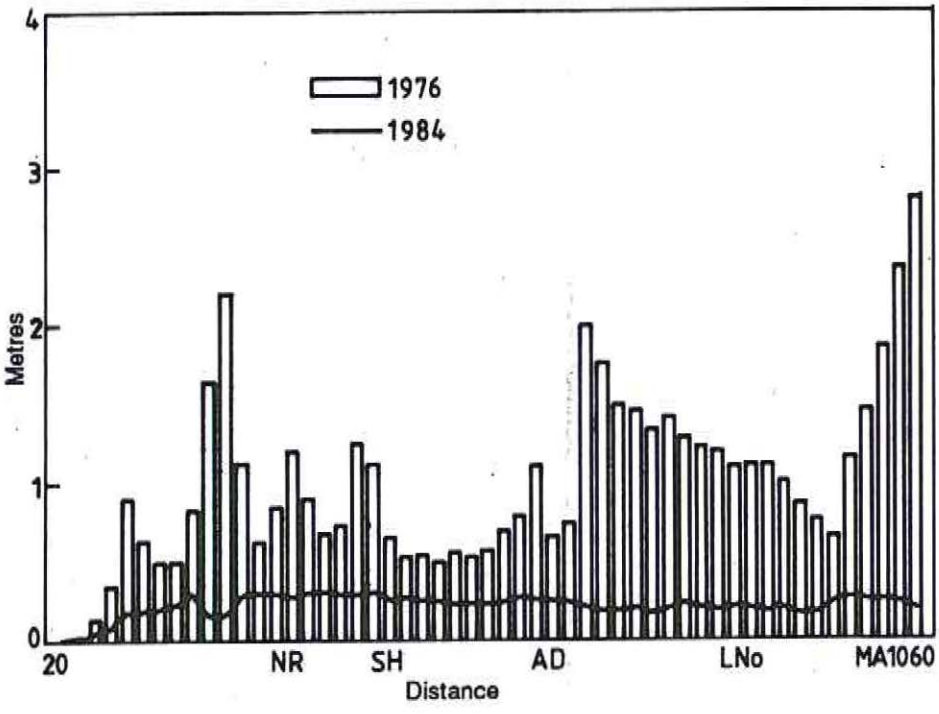

Figure 5. Density and distribution of floating hyacinth January–February 1976 and 1984.

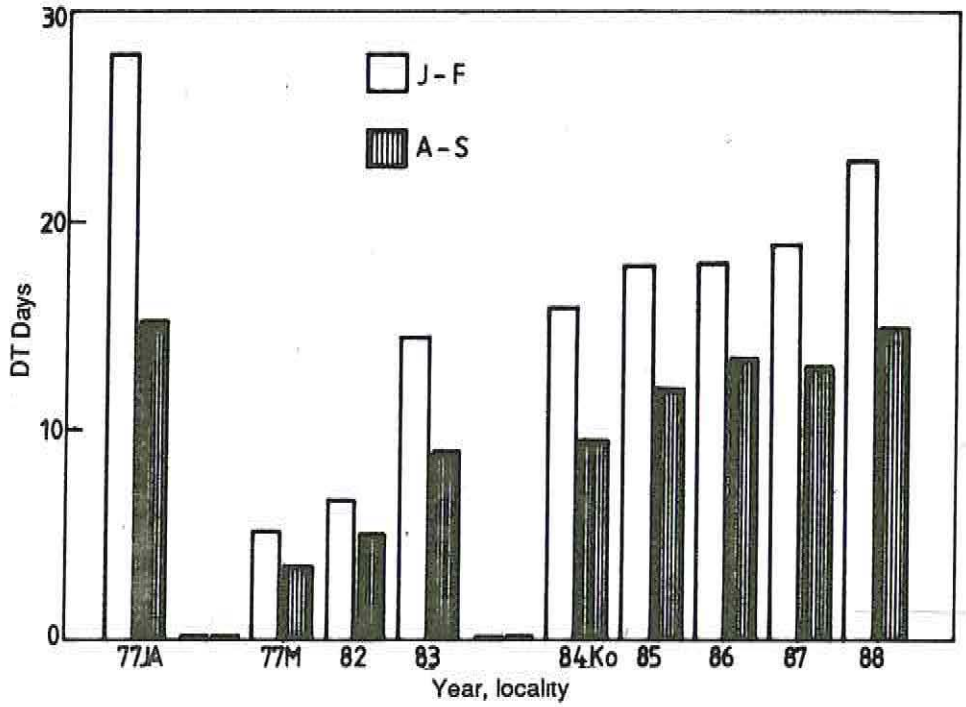

Figure 6. Doubling time of field WH in various localities 1977–1988.

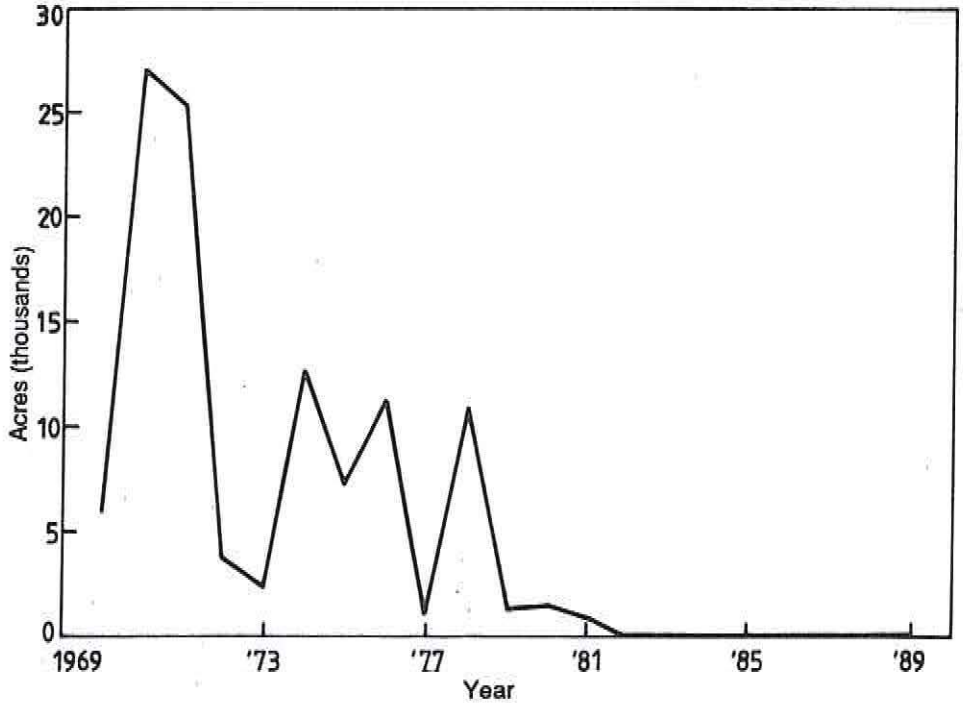

Figure 7. Water hyacinth observed behind Jebel Aulia Dam 1969–1989.

Results on the productivity of water hyacinth under field conditions at Jebel Aulia (JA), Malakal (M) and Kosti (KO) areas are presented in Fig. 6. These results show the doubling time of water hyacinth under density dependent conditions before and after the release and establishment of biocontrol agents. In Malakal area the doubling time increased for 5 and 3 days in January–February and August–September respectively 12 and 10 days in the same respective months in 1983. Progressive increase in the doubling time is noticed in Kosti area from 1984 to 1988. This is an indication of a build up of suppression by the natural enemies leading to reduction in the productivity of the plant in that area.

Readings obtained on the total area of hyacinth that annually accumulated behind the Jebel Aulia Dam presented in Fig. 7 showed that a maximum of 27,000 acres of hyacinth was reported in 1970 and a minimum of 4000 in 1977. Since 1980 few plants were noticed behind the dam. Starting from 1982 up to 1988 virtually no hyacinth was recorded behind the dam. The dam being a physical barrier annually received the surplus of hyacinth drifting from the discharge areas in the sudd region during the rainy season after the side channels and oxbows are filled. Again this marked decrease in the amount of surplus hyacinth is an indication of a marked reduction in the productivity of the plant.

Since 1983 very little effort was made to control water hyacinth by the herbicide 2,4 D. In spite of this there was marked reduction in its population, evident from data obtained in the breeding sites in the sudd area and the ultimate discharge and accumulation site behind Jebel Aulia dam. It is true that there were annual fluctuations in the population density of the plant since it has established itself in that area. This is evident from the data on the hyacinth that accumulated behind the dam (Fig. 7) and data obtained by Freidel (1979) on both the bank and floating hyacinth. These fluctuations are caused by fluctuations in the physical factors particularly rainfall, wind speed and the height of the flood that markedly affected the density and spatial distribution of the plant. Nevertheless water hyacinth never completely or markedly disappeared from behind the Jebel Aulia Dam during the rainy season in any one year like it did now and since 7 years starting from 1982 to 1989. During this period the only new factor was the establishment and association of introduced biocontrol agents with the plant in all its domain from Juba northwards up to Kosti.

Further analysis of data on the density and damage by the introduced natural enemies on water hyacinth are being made. Data on

the correlation between population density of the natural enemies and their damage, meteorological factors and other noticed results such as increase in the discharge of the various White Nile tributaries and the recess in the population density and spatial distribution of water hyacinth are being accumulated and analyzed.

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Bacillus thuringiensis: The Killing Factors of a Multiphage-resistant Mutant in the Silkmoth, *Hyalophora cecropia*

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Bacillus thuringiensis: The Killing Factors of a Multiphage-resistant Mutant in the Silkmoth, *Hyalophora cecropia*

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INTRODUCTION

In microbial control of insect pests, *Bacillus thuringiensis* has been so far the bacterial agent widely used. The insecticidal properties of *B. thuringiensis* are mainly due to inclusions consisting of proteins active against larvae of lepidoteran, dipteran or even coleopteran species (Hofte and Whiteley, 1989).

Besides the production of toxins, other factors contribute to the virulence of *B. thuringiensis* in insects. These include the production of two immune inhibitors as well as resistance to immune systems of insects (Edlund *et al.*, 1976; Boman and Steiner, 1981). It has shown that when insects are injected with viable bacteria, they develop a cell-free antibacterial activity (Chadwick and Aston, 1979). In the silkmoth *Hyalophora cecropia* immunity has been induced by challenging diapausing pupae with a moderate dose of viable *Enterobacter cloacae* and other non-pathogenic bacteria (Faye *et al.*, 1975). The vaccination induces the synthesis of about 15 bactericidal proteins divided into three groups; the cecropins, the attacins and lysozyme (Boman and Steiner, 1981; Hultmark *et al.*, 1983). The cecropins are basic polypeptides with activity against a wide range of gram-negative and gram-positive bacteria (Hultmark *et al.*, 1982). The attacins act against a narrow range of susceptible bacteria and kill only growing cells (Hultmark *et al.*, 1983), while lysozyme is effective against a few gram-positive species (Steiner *et al.*, 1981).

Haemolymph from immunized *H. cecropia* pupae has been demonstrated to have an antibacterial activity of low level against wild type *B. thuringiensis* (Edlund *et al.*, 1976). The bacterium, however, grew in haemolymph from unimmunized pupae. The bacterium was also highly resistant to purified cecropins, attacins and lysozyme tested singly or in pools. These results taken together raise the possibility that other unidentified factors in the immune haemolymph (IH) of *H. cecropia* have an anti-*B. thuringiensis* action.

In an attempt to study the unidentified factors, a multiphage-resistant mutant with a defective petridoglycan and decreased passive resistance to IH (Kivaisi, 1985) was used. In the study, the factors have been preliminarily characterized.

MATERIALS AND METHODS

Bacterial strains

Bacterial strains used and their sources are shown in Table 1.

Table 1. Bacterial strains used

Organism	Strain	Parent	Subspecies	Source/Reference
*B.t.**	Bt 1003	Bt 1	*Gelechiae*	Kivaisi (1985)
B.t.	Bt 1013	Bt 1003	*Gelechiae*	Kivaisi (1985)
E. cloacae	B 12	–		Boman *et al.* (1974)
E. coli	D 31			"
B. cereus	Bc 11			Edlund *et al.* (1976)

**B. thuringiensis.*

Insects Used and Procedure for Immunization

Diapausing pupae of the silkmoth, *Hyalophora cecropia* were used. They were obtained from Shelton's U.S.A. and stored at 8°C. During the experimental period, the pupae were maintained in a climate chamber (Shever CEL 44) at 25%, 7% Relative Humidity (RH) and 15 hr light. Immunization was performed by injecting viable cells of *Enterobacter cloacae*, strain B12 into the thoracic region of the pupae at a dose of about 10^6 cells per pupa using the method of Boman *et al.* (1974) and after 3–34 days the immune haemolymph was collected.

Media and Other Chemical Used

Liquid medium (LEG) in the form of LB medium (Bertani, 1951) supplemented with medium E (Vogel and Bonner, 1956) and 0.2% (w/v) glucose and solid medium (LA) in the form of LB medium solidified with 1% (w/v) agar were used. All dilutions were performed with dilute nutrient broth (DIL) 0.1% (w/v) containing NaCl (0.5%, w/v); potassium phosphate buffer (pH 7.4) contained in g/l K_2PO_4, 100; K_2HPO_4, 46; and the pH was adjusted with 0.1 M NaOH.

Trypsin was obtained from Sigma Chemical Company, St. Louis, U.S.A. Inhibitor B was kindly supplied by Jong-youn Lee of the Department of Microbiology University of Stockholm, Sweden.

Antibacterial Activity Assay

The killing assay: A reaction mixture containing 90% IH and about 5×10^2 viable log-phase cells was incubated for 2 hr at 22°C. 5 µl samples were withdrawn at 30 min intervals and plated onto LA. These were incubated overnight at 37°C, after which surviving cells were scored and percentage survival calculated.

Inhibition zone assay (plate assay): The agar mixture contained about 105 cells of the test bacterium and 5 ml of LA supplemented with 1 µg/ml penicillin-G and 0.25 mg/ml^{-2} phenylthiourea. The mixture was spread on 9 cm plastic petri-dishes on a levelling table. 2.0 µl of the IH sample were pipetted into 2 mm diameter wells in the agar. The plates were incubated for 15 to 20 hr at 37°C. The diameter of the zones of growth inhibition were measured as described by Hultmark *et al.* (1980).

Experiments: Immune haemolymph was incubated with different concentrations of trypsin for 30 min at 37°C and assayed for anti-*B. thuringiensis* activity in a plate assay. In another experiment, IH was treated with inhibitor B (Edlund *et al.*, 1976) at a final concentration of 25%. The mixture was incubated in a shaker water-bath at 37°C for 30 min and assayed for the anti-*B. thuringiensis* activity in a plate assay. The activity was also assayed in dialyzed IH. The haemolymph was dialyzed for 48 hr against 0.1 M potassium phosphate buffer (pH 7.4) at 5°C and then tested by a plate assay.

The antibacterial activity against *B. thuringiensis* was followed for 8 days in pupae injected with *E. cloacae*, B12 or Lepidoptera ringer. Haemolymph samples (25 µl) each were withdrawn from the pupae at different time intervals after injection and were tested for antibacterial activity against *B. thuringiensis* (Strain Bt 1013) and *Bacillus cereus* (strain Bc 11) in a killing assay.

RESULTS

Characterization of anti-*B. thuringiensis* activity

When the multiphage-resistant mutant, strain Bt 1013 and its parent, strain Bt 1003 were tested for susceptibility to IH in the presence of penicillin-G (1 µg/ml) in a killing assay, all cells of both strains were

killed in 60 min. In the absence of penicillin, the IH killed all Bt 1013 cells in 120 min, whereas, Bt 1003 cells were not killed (Fig. 1a and 1b).

While activity against *Escherichia coli* strain D31 was eliminated at 250 µg/ml of trypsin, the anti-*B. thuringiensis* activity was reduced by 50% at 100 µg/ml of the enzyme and was not eliminated at higher concentration (Fig. 2). The activity in the IH against *B. thuringiensis* was reduced not only by inhibitor - B, but also by dilution with potassium phosphate buffer (Table 2). Dialysis of the IH against potassium phosphate buffer (pH 7.4) for 48 hr reduced the activity by 35% (Table 3). Figure 3 shows that the anti-*B. thuringiensis* activity almost disappears from the IH when diluted 8 times, while the activity against *E. coli* had to be diluted by more than 32 times before it disappears (Faye, 1976).

Induction kinetics of the anti-*B. thuringiensis* activity

The induction kinetics of the activity against *B. thuringiensis* in immunized pupae of *H. cecropia* were compared to that of its close relative *B. cereus*. The anti-*B. thuringiensis* activity was first detected during the 24th hour and reached a maximum on the 4th day after immunization. The anti-*B. cereus* appeared after 48 hr and reached a maximum 3 days later (Fig. 4). In a control experiment with saline injected pupae (results not shown), activities against the two bacterial strains were lower than those induced by immunization with *E. cloacae*,

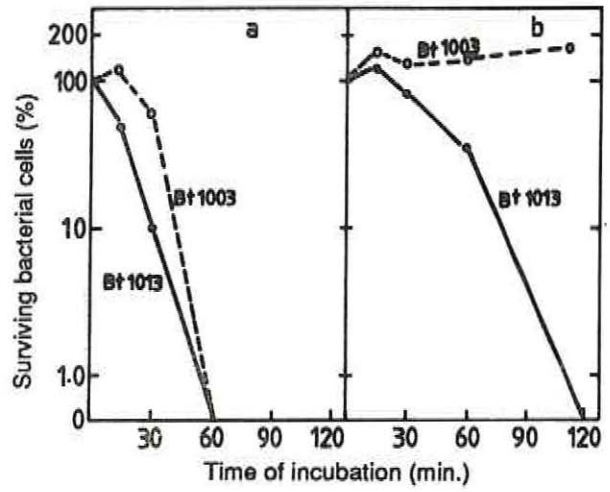

Figure 1. Surviving cells of Bt 1003 (open symbols) and Bt 1013 (filled symbols) after incubation with immune haemolymph of *H. cecropia* pupae. The reaction mixture contained 50 µl of haemolymph and 5 µl containing about 5 x 10^2 viable bacteria with penicillin (a) and without penicillin (b).

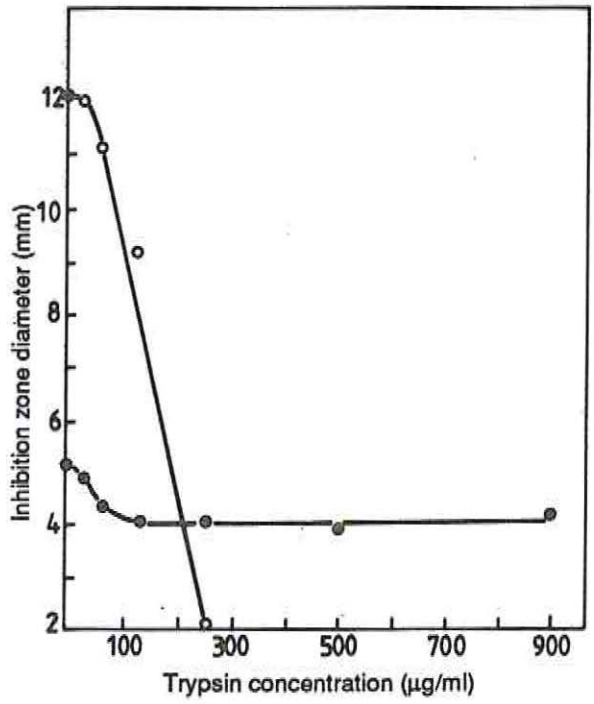

Figure 2. The antibacterial activity against *E. coli*, strain D31 (open circles) and *B. thuringiensis*, strain Bt 1013 (filled circles) in the immune haemolymph of *H. cecropia* after incubation with different concentrations of trypsin for 30 min at 37°C. The activity was recorded as inhibition zone on thin agar plates as described in Materials and Methods.

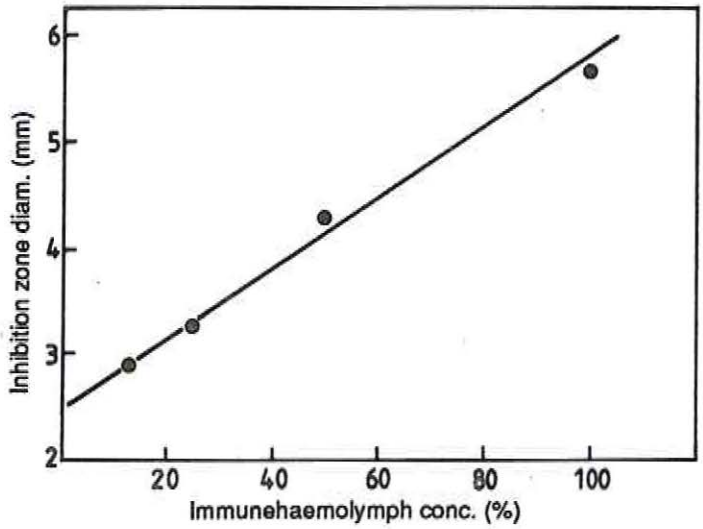

Figure 3. A reference for the anti-*B. thuringiensis* activity present in immune-haemolymph of *H. cecropia*. The curve is based on an average of three tests with Bt 1013 as an indicator strain in a plate assay.

Table 2. Antibacterial activity[1] in Inhibitor B (InB) treated immune haemolymph of *Cecropia* pupae against the phage resistant mutant (Bt 1013), the parent (Bt 1003) and the wildtype (Bt 1)

	Diameter of inhibition zone in mm			
Strain	NH	IH	IH + InB	IH + buffer
Bt 1	2.0[2]	2.9	2.7	2.7
Bt	2.0	2.8	2.1	2.5
Bt 1013	2.0	4.5	3.7	3.4

[1] Antibacterial activity was recorded as inhibition zones on thin agar plates as described in Materials and Methods. Normal haemolymph (NH) and immune hemolymph (IH) were included as references. The concentrations of InB and buffer (0.1 M potassium phosphate buffer, pH 7.4) were 25% respectively.
[2] The diameter of the well was 2.0 mm.

Table 3. Antibacterial activity[1] in dialyzed immune haemolymph of *H. cecropia* pupae against the multiphage resistant mutant, Bt 1013

	Diameter of inhibition zone in mm		
Test	NH	IH	Dialyzed IH
1	2.0[2]	6.0	4.7
2	2.0	6.3	5.0
3	2.0	6.0	4.3

[1] Antibacterial activity was recorded as inhibition zones on thin agar plates as described in Materials and Methods. Normal haemolymph (NH) and immune haemolymph (IH) were included as controls. The test haemolymph was dialyzed against 0.1M potassium phosphate buffer, pH 7.4.
[2] The diameter of the well was 2.0 mm.

and appeared much later.

DISCUSSION

The results of this study have demonstrated that *B. thuringiensis* killing factors present in the IH of *H. cecropia* pupae act synergistically with penicillin-G. The target of all or some of the factors could be the cell wall. The indicator strain (Bt 1013) has been shown to have a defective peptidoglycan (Kivaisi, 1985) which could easily be attacked by penicillin and the active factors in the haemolymph. The failure of the normal haemolymph (NH) to kill the parent (Bt 1013) strains in the presence of penicillin indicates that the killing factors are previously non-existent in the haemolymph but are inducible.

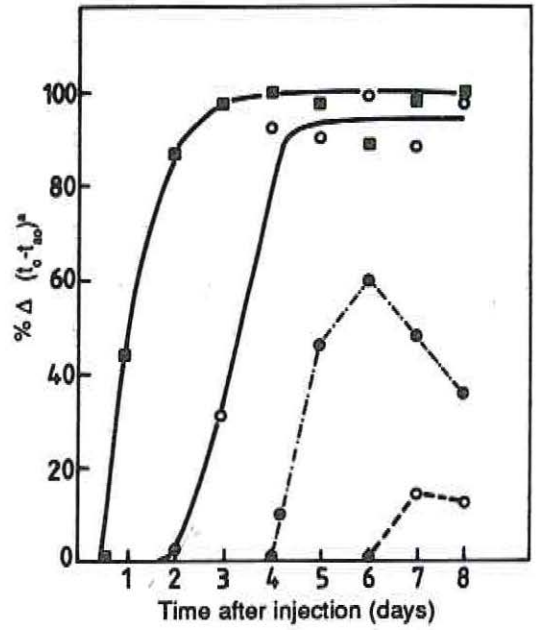

Figure 4. Induction kinetic curves for the antibacterial activity against Bt 1013 (half filled squares) and Bc 11 (open circles) in haemolymph from *H. cecropia* pupae immunized with β 12, and from pupae injected with Lepidoptera ringer against Bt 1013 (filled circles) and Bc 11 (open circles). The reaction mixture contained 25 µl haemolymph and 2.5 µl containing about 5 x 10^2 bacteria and 1 µg/ml penicillin. *% (t_o t_∞) = percent bacterial cells killed after 80 min of incubation with haemolymph.

A multi-component system has been shown to be associated with anti-*B. thuringiensis* activity in the haemolymph of locusts with anti-*B. thuringiensis* activity in the haemolymph of locusts (Hoffman, 1980). The results reported here show that the anti-*B. thuringiensis* activity in *H. cecropia* was not interfered with by inhibitor-B. Instead, it diminished either on dialysis or on application of trypsin. These results indicate the involvement of a multi-component system in this moth as well.

It was observed that the anti-*B. thuringiensis* activity disappeared from the IH of *H. cecropia* when diluted 8 times. It was earlier reported by Fare (1976) that the antibacterial activity against *E. coli* in *H. cecropia* has to be diluted 32 times before disappearance from the IH. This implies, therefore, that the killing factors produced by the *H. cecropia* moth against *B. thuringiensis* are of smaller concentrations in comparison with the factors produced against *E. coli*.

Observations from experiments on induction kinetics indicate that activities against *B. cereus* and *B. thuringiensis* appear at different times

after immunization. It may be concluded that either the two species are killed by different factors or they differ in sensitivity to the induced factors. The latter conclusion is most likely since *B. cereus* is a close relative of *B. thuringiensis*.

From the overall results, it may be concluded that anti-*B. thuringiensis* activity in *H. cecropia* arises from a gradual induction of complex, relatively diluted factors in the haemolymph. These factors are different from those reported earlier (Boman and Steiner, 1981; Hultmark *et al.*, 1983).

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Highlights and Future of Biological Control Research in Ghana

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Highlights and Future of Biological Control Research in Ghana

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INTRODUCTION

Plant pests and diseases are about the most important factors hampering food production in Ghana. Pre and post harvest losses attributable to pest and diseases alone are estimated at 40–55 percent (PPRS, 1989).

The government of Ghana in recognition of the agricultural potentials of the country, has recently intensified its drive to improve the Agricultural Sector. This has brought about improving the use of scientific farming practices, which create suitable conditions for an upsurge in pestilence from both indigenous and exotic pest organisms. The recent invasion of the country by two cassava pests; the cassava mealybug (CM) *Phenacoccus manihoti* and the cassava green spider mite (CGM) *Mononychellus tanajoa* and lately the mango/plantain mealybug *Rastrococcus invadens* and the upsurge in recurrence of indigenous pests as the oil palm leaf miner beetle *Coelaenomenodera alaeidis* and the variegated grasshopper *Zonocerus variegatus* are ample testimony.

Despite the efforts of the Commonwealth Institute of Biological Control (CIBC) West African Substation (which was established in Kumasi under the auspices of the Crops Research Institute (CRI) in 1969, and was converted to the Biological Control Division of the CRI in 1979 following the withdrawal of the CIBC and classical biological control of indigenous pests, pest control in Ghana, until recently, relied heavily on the use of chemical pesticides. Little attention was hitherto paid to biological control, probably because of high initial capital inputs. The use of modern chemical pesticides is known to entail numerous problems (Stern *et al.*, 1959), particularly in less technologically developed countries like Ghana.

Two new cassava pests, the cassava mealybug *P. manihoti* and the cassava green spider mite *M. tanajoa* were discovered in 1980 (Korang-Amoako *et al.*, 1987). Their subsequent devastation of the cassava agro-ecosystem resulted in yield losses of about 70 percent (PPRS, 1989).

Following the failure of the Plant Protection and Regulatory Services to secure their control through conventional control methods as quarantine, chemical sprays and cultural practices and as though drawing inspiration from Lewis *et al.* (1982), who summed up the pest control challenge of the future as the development of techniques for augmenting and manipulating the performance of natural enemies; in other ways enhancing the benefit of natural weapons or somehow artificially intervening with selective non disruptive measures, an Integrated Pest Management approach with biological control as its backbone was adopted.

This paper reports on the collaborative activities of the Biological Control Division in finding suitable controls for the cassava mealybug, the cassava green spider mite and the mango and plantain mealybug, general research efforts at developing suitable alternatives to conventional pesticides in an IPM context and the long term prospects of biological control on various pest organisms in Ghana.

CURRENT WORK

Biological Control of the CM, CGM and Mango Mealybug

Following the invasion of the African cassava ecosystem by the cassava mealybug *P. manihoti* and the green spider mite *M. tanajoa* (Herren *et al.*, 1985), and their discovery in Ghana in 1980 (Korang-Amoako *et al.*, 1987), the Division entered into collaboration with the Biological Control Programme (BCP) of the International Institute of Tropical Agriculture (IITA) (currently stationed in Benin), the Plant Protection and Regulatory Services (PPRS), in which Ghana was to control the two pests. The Division, together with the PPRS engage in pre-release surveys to identify suitable release fields, receive and release on the ground parasites and predators from the BCP of IITA. After releases have been made, post release monitoring is done to ascertain the spread and efficacy of released parasitoids and predators. Studies are also carried out on the interrelationships between the pests and local fauna (natural enemies).

Both ground and aerial releases of natural enemies were made against the two pests in 1984 and 1985. The Encyrtid parasitic wasp *Epidinocarsis lopezi* and the predators *Diomus* spp., and *Hyperaspis* spp. as well as *Sympherobus* spp. were released against the cassava mealybug at Pokuase, Sege, Ohawu, Legon, Kwadaso, Akotokyere and New Tafo among other places. The predatory mites *Amblyseius* spp., *Neoseiulus ideaus* and *Typhlodromalus limonicus* were released against the cassava

green spider mite in various locations. Since then, various releases of predatory mites have been made in locations in the various ecological zones in Ghana (Korang-Amoako *et al.*, 1987).

Studies on Biological Control of the Mango Mealybug

The mango mealybug *Rastrococcus invadens*, was first recorded in Ghana in 1982 (PPRS, 1989). It is a typically polyphagous insect attacking citrus, plantains and bananas, avocado, pear, guava as well as many ornamental crops and vegetables. Yield losses of about 80 percent were observed on attacked trees. Studies on the biology of the mealybug revealed that it carried its eggs below the abdominal segments unlike *P. manihoti* where egg sacs are displayed. Males in the mealybug unlike the cassava mealybug are very common and were found to be highly phototactic. Coccinellid beetle predators of *Rastrococcus* were collected in surveys in the coastal savanna, and the forest zones of Ghana. Key among them were *Chilocorus nigrita, Exochomus promtus*, and *Exochomus troberti*. These local fauna associated with the mealybug did not cause any recognizable reduction in population. In August 1988, the BCP of IITA supplied the parasitic wasp *Byranusoidea tebygi* for ground releases against *Rastrococcus*.

Evaluation of Dipel 2x for the Control of Lepidopterous Pests

The Division obtained samples of Dipel 2x (*B. thuringiensis*) from Abbot Laboratories of the U.S.A. to test against various lepidopterous pests such as the cowpea pod borer, *Maruca testulalis*, the cabbage leaf worm, *Spodoptera littoralis* and the egg plant shoot and fruit borer, *Leucinodes orbonalis*.

Biological Control of the Siam Weed

Recently (1987), the Division in a collaborative effort with Professor Maniappan of the Agricultural Experiment Station Mangilao Guam took delivery of *Pareuchaetes pseudoinsulata* for the biological control of the Siam weed, *Chromolaena odorata*. Breeding of the insect in the laboratory has reached the third generation. Host specificity tests have been carried out on cocoa, *Theobroma cacao*; oil palm, *Elaeis guineensis*; gauva, *Psidium guajava*; citrus, *Citrus auriantifolia*; mango, *Mangifera indica*; maize, *Zea mays*; cowpea, *Vigna unguiculata*; cabbage, *Brassica oleracea*; egg plant, *Solanum melongena* and cassava, *Manihot esculenta*.

So far all crops tested have been rejected even though the larvae were supplied fresh samples until they died. Field release would be made when substantial numbers have been bred.

Effect of Spray Arrangement of Recommended Insecticides for Control of Field Pests of Cowpea

The objective of this project is to determine suitable spray arrangements of recommended cowpea insecticides to approximate conventional 'blanket' sprays in effectiveness and yet reduce expenses in cowpea production and harm to beneficial organisms. Cymbush is used as a preflowering insecticide while Roxion is applied post flowering.

RESULTS

Biological Control of Cassava Pests

Post-release monitoring, after 3 months of release of *Epidinocarsis lopezi*, revealed the establishment at Ohawu, Pokoase and Sege and spread to other areas. Local beneficial insects mainly *Exochomus* spp., *Hyperaspis* spp., and *Dicrodiplosis* spp. were found associated with the mealybug (Korang-Amoako *et al.*, 1987). Monitoring and evaluation has so far proved that *E. lopezi* has been effective in suppressing the population of *P. manihoti*.

The predatory mites released against the cassava green spider mites did not establish. However, the search for more suitable species continues at IITA. The locally available predatory phytoseiid mite, *Amblyseius* spp., observed in association with *M. tanajoa*, does not appear effective as a natural enemy. It is suspected this is because it is polyphagous and not specific to *M. tanajoa*.

Biological Control of Mango Mealybug

Monitoring and evaluation after the release of *G. tebygi* revealed that the parasite had established and had effectively suppressed *R. invadens*. So fantastic has been the result that the farming public has began to appreciate the potential of biological control. A drastic improvement in mango yields has been recorded since the establishment of the parasite (PPRS, 1989).

Evaluation of Dipel 2x for the Control of Lepidopterous Pests

Dipel 2x did not result in increased seed yield over controls where a

blank solution was applied. Results were generally inconclusive and this was attributed to the numerous non-caterpillar pests of post flowering cowpea.

Dipel 2x was also out performed when it was tested against Karate 2.5EC in the control of the egg plant shoot and the fruit borer, *Leucinodes orbonalis*. There were no differences among the concentrations of Dipel 2x tested in fruit yield, number of flower buds infested, number of fruits infested and number of exit holes per fruit. When Dipel 2x was tested against Karate 2.5EC to assess its ability to cause mortality in the cabbage leaf worm *Spodoptera littoralis*, none of the five concentrations of Dipel (2g/l, 1g/l, 0.5g/l, 0.25g/l and 0.125g/l) could effect any kill after 24 hrs. Karate 2.5EC effected a 100 per cent kill within the same period.

The above results cast serious doubts on the potency of Dipel 2x. It is suspected that poor storage which probably led to rapid deterioration of the samples and low concentrations of the active ingredient in the doses tested, could be reason for the results. The tests will be repeated at higher concentrations of Dipel 2x to verify the authenticity of the results.

Biological Control of the Siam Weed

Releases of *P. pseudoinsulaia* are yet to be made in the field. Until releases have been made and post release monitoring carried out, no conclusions can be drawn.

Effect of Spray Arrangement of Recommended Cowpea Insecticides for Control of Field Pests of Cowpea

Analysis of the field data obtained showed that no significant differences existed in mean count of flower thrips *Megalurothrips sjostedti* and seed yield between the insecticidal spray arrangements tested. However, all insecticidal arrangements resulted in significantly higher yield and low thrips count than the control where no insecticide was applied at all. The non significant differences were probably attributable to insecticidal drift.

FUTURE PROSPECTS OF BIOLOGICAL CONTROL IN GHANA

With the impact of the results of the collaborative work with IITA BCP on the biological control of the cassava mealybug and the mango

mealybug, *R. invadens*, the future is bright for biological control in Ghana.

The Biological Control Division will continue to collaborate with IITA BCP and other agencies in an effort to find suitable controls to the cassava and mango pests as well as other identifiable pest situations.

The biological control project initiated for the Siam weed will be pursued vigorously. Further work on Dipel 2x (*B. thuringiensis*) will be carried out at increased concentrations. Other alternatives to *B. thuringiensis* will be sought and tested.

It must be stressed that the future of effectively organizing any biological control programme in Ghana depends on the availability of sufficient finances. Probably the IITA BCP has managed successful controls of CM and *R. invadens* because their activities are adequately funded and thus well organized. Where funds are available candidate pests with biological or integrated pest management potentials include:

(i) the variegated grasshopper *Zonocerus variegatus* (Orthoptera: Acrididae) which is a polyphagous and endemic pest in Ghana.

(ii) the oil palm leaf miner beetle *Coelaenomenodera elaeidis* (Coleoptera: Hsipidae) which is a serious pest of oil palm in Ghana. Two major cyclical outbreaks have been recorded in the last 10 years. Though this Division has been offering advice on the pest since 1974, lasting control is yet to be achieved. Advice emphasizing an integrated approach and discouraging the massive application of insecticides has been neglected. Despite the implementation of a biological control programme in nearby La Cote d'Ivoire (Decker pers. com.) lack of funds has made it impossible for any advantage of such a programme to be taken. There is presently an urgent need for funds to implement a biological control project on *C. elaeidis* following a recent outbreak (Feb. 1990).

(iii) The larger (greater) grain borer — *Prostephanus truncatus* (Coleoptera: Bostrichidae). The latest threat to agriculture, especially farm stored maize in Ghana, is *P. truncatus* (Dixon pers. com.). It also attacks dried cassava and fish in storage. It is feared to cause damage to wooden structures (GASGA, 1987). Losses of 70 percent have been reported after only 4 months in storage (GASGA, 1987). Benz (1986) reported that *P. truncatus* responds to dominicalure 1 and 2, a male produced aggregation pheromone. Pheromone traps are already being used to monitor the populations of *P. truncatus* in the Eastern borders of

Ghana (Dixon, pers com.). Apart from cooperating with the IITA BCP in organizing a regional biocontrol programme for the pest, the possibilities of developing an integrated pest management package through studies on pheromones, and chemicals with juvenile hormone activity e.g. Varikill (Phenoxy-ethyl — Carbamate) as well as various storage techniques will be investigated.

(iv) Biological Control of water weeds (water lettuce — *Pistia* sp., water fern *Salvinia molesta* and water hyacinth *Eithornia crassipes*). These weeds have become serious problems in our fresh water bodies. Various efforts are being made at their control. However, it is envisaged that the most promising tool for their control is classical biological control as they are invariably foreign.

CONCLUSION

A great potential exists for biological control in Ghana. Drawbacks to the realization of this potential are inadequate funding for biological control research and lack of trained manpower.

The initial costs of organizing a biological control programme is probably beyond the capability of any individual African country. Regional groupings of countries to tackle common problems are most advisable. Because such groupings are more viable as they stand a chance in attracting international assistance.

The present collaborative work with various agencies can only be meaningful if adequate facilities exist for national scientists to continue when these agencies leave. It will therefore be recommended that the national collaborating groups be strengthened financially and otherwise to reduce the present total dependence on the BCP of IITA.

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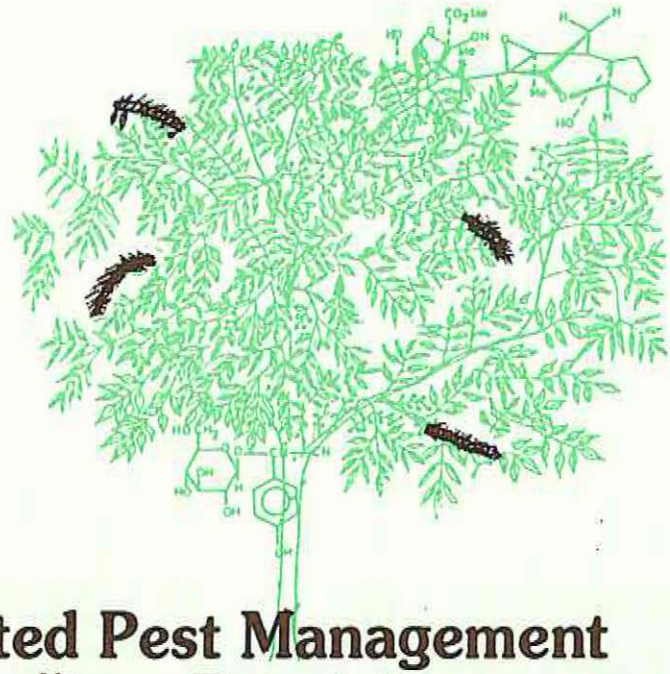

Integrated Pest Management of Whiteflies, *Bemisia* Spp. in Malawi

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Integrated Pest Management of Whiteflies, *Bemisia* Spp. in Malawi

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INTRODUCTION

Whiteflies, especially *Bemisia tabaci*, occur on a wide range of wild and cultivated plants. They are known to damage crops directly, by removing plant sap, and indirectly through the growth of black mould on the honeydew which they exude onto the top surfaces of lower leaves and through the introduction of viruses. It is suspected that the whitefly transmits virus diseases to crops such as tomatoes, some vegetables, tobacco, cassava, European potatoes and sweet potatoes. However, no research data is available to confirm this role on most of these crops in Malawi. The *Bemisia* species complex that exists in Malawi and its natural enemies have not all been identified and the population dynamics of the whitefly in Malawi are also not well understood.

This paper summarizes the available information on the past status of the whitefly on cassava in Malawi. It is hoped that an understanding of the biology, distribution and population dynamics of the whitefly and its natural enemies will enable the development of an integrated control strategy against the pest. Cassava was selected because it is the second major food crop in Malawi, and because the whitefly is reputed to transmit the African cassava mosaic virus, one of the most serious production constraints for this crop. In this report, a brief summary of the work undertaken in Malawi so far has been presented and the implications of the results discussed.

NATIONAL SURVEY

The objective of the survey was to establish the distribution of the

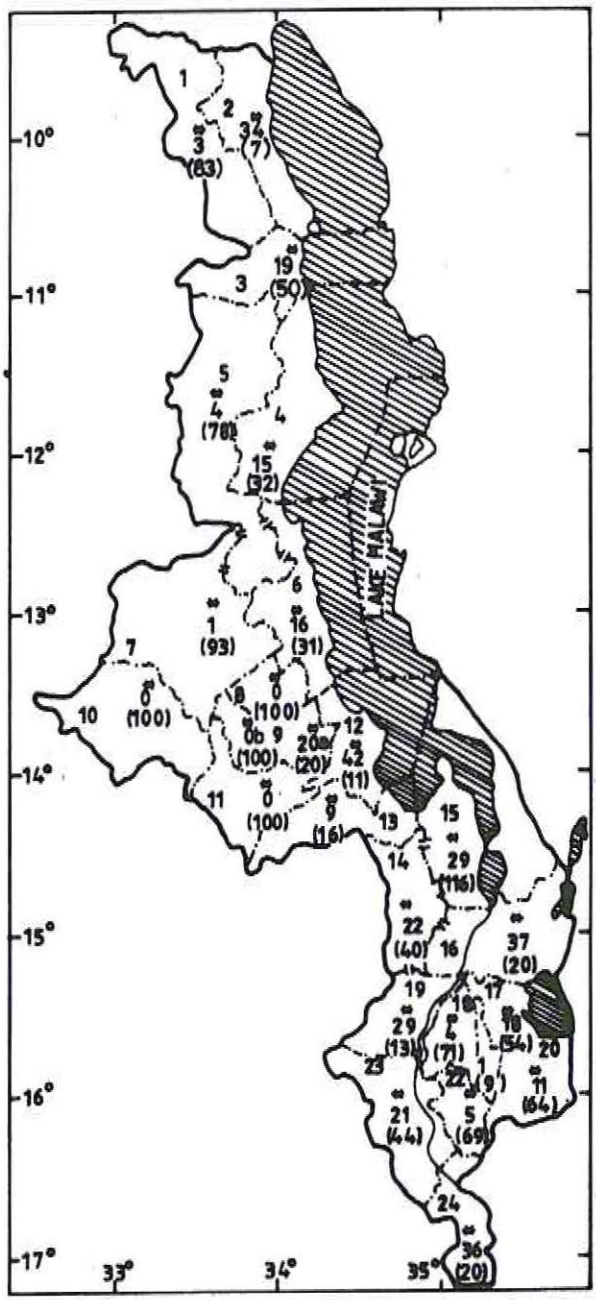
whitefly and its parasitoids, assess levels of incidence of the cassava mosaic virus disease at different altitudes, and identify cassava varieties which could be used in an integrated pest management programme against the whitefly. The survey was also aimed at obtaining farmers' opinions on the status of cassava in their farming system, as well as their perception of the whitefly and the African cassava mosaic virus disease (CMVD). The survey revealed that cassava is grown throughout the country. In areas where cassava is not a staple, many farmers grow it as a security crop to provide them with food in times of maize failure. Some grow the crop as a cash crop at the village level or for sale in markets. Both *B. afer* and *B. tabaci* were found to infect cassava throughout the country, with *B. afer* the more prevalent. Several predators and parasites were found to attack the whitefly in Malawi.

The CMVD was found to occur throughout the country. Most farmers were aware of its destructive effects. However, a few thought that the plants which exhibited the CMVD symptoms were actually different varieties from those which did not. Although in some cases the disease was present because infected planting material had been used, in many cases the farmers indicated that they had initially used clean planting material and that the plants were infected later during growth. This emphasizes the importance of the whitefly as the vector of the CMVD.

High altitude areas generally had low incidences of the CMVD (Fig. 1 and Table 1). The incidence of diseased plants in areas above 800 m was less than 5% while most fields in sites below 800 m had an incidence of between 15% and 70% on average. Infection rate as high as 90% were in some low land fields. A number of varieties were observed to be tolerant to the CMVD indicating that local resistant material could play an important role in controlling the disease.

RATE OF CMVD TRANSMISSION IN THE FIELD

In this study, a number of varieties commonly grown in Malawi were tested in areas above 800 m and below 800 m. Material which had shown CMVD symptoms for the previous 12 or 24 months was planted in a randomized block design. A row of cassava with CMVD symptoms was planted between treatments to act as a CMVD reservoir and source. The objective was to identify varieties which did not acquire the CMVD rapidly in the field and formulate recommendations on how frequently farmers need to replace their planting material with clean materials if they use specific cassava varieties. The planting material used in this



Northern Region

1. Chitipa
2. Karonga
3. Rumphi
4. Nkhata Bay
5. Mzimba

Central Region

6. Nkhotakota
7. Kasungu
8. Ntchisi
9. Dowa
10. Mchinji
11. Lilongwe
12. Salima
13. Dedza
14. Ntcheu

Southern Region

15. Mangochi
16. Machinga
17. Zomba
18. Blantyre
19. Mwanza
20. Mulanje
21. Chiradzulu
22. Thyolo
23. Chikwawa
24. Nsanje

Key

% ACMV (African cassava mosaic virus)

( ) % fields clean

*a Part of Dowa District under Salima Agricultural Develop. Division

*b Part of Dowa District under Kasungu Agricultural Develop. Division

Figure 1. The percentage of cassava fields free from CMVD and the percentage CMVD infestation per field in different districts of Malawi.

Table 1. Mean % CMVD and fields free from CMVD in districts of Malawi

District	Agricultural Development Division	No. of fields sampled	% fields free from CMVD	CMVD % field
Chitipa	Karonga (KRADD)	18	83	3
Karonga	KRADD	28	7	34
Mean		23	45	19
Rumphi	Mzuzu (MZADD)	8	50	19
Mzimba	MZADD	18	78	4
Mkhata Bay	MZADD	22	32	15
Mean				
Kasungu	Kasungu (KADD)	14	93	1
Mchinji	KADD	6	100	0
Ntchisi	KADD	5	100	0
Dowa	KADD	5	100	0
Mean		7.50	98.3	0.25
Lilongwe	Lilongwe (ADD)	16	100	0
Dedza	LADD	19	16	9
Ntcheu	LADD	15	40	22
Mean		17	52	10.2
Mkhota kota	Salim ADD (SLADD)	26	31	16
Salima	SLADD	16	11	42
Dedza	SLADD	3	0	70
Dowa	SLADD	5	20	20
Mangochi	SLADD	3	0	60
Mean		11	21	41.6
Machinga	Liwonde ADD (LWADD)	46	20	37
Mangochi	LWADD	26	43	27
Zomba	LWADD	26	54	18
Mean		32.7	39.1	30.20
Blantyre	Blantyre (BLADD)	7	71	4
Mwanza	BLADD	15	13	29
Chiradzulu	BLADD	11	91	1
Thyolo	BLADD	13	69	5
Mulanje	BLADD	26	75	36
Mean		14.4	63.8	15.00
Chikwawa	Ngabu ADD (NADD)	9	44	21
Nsanje	NADD	5	20	36
Mean		7	32	28.50
Country mean		16.8	49.9	19.9

Table 2. Percentage of plants with CMVD 12–14 months after planting

	Makoka	Chitedze	Chitala	Baka	Mkondezi	Mean
Chitembwere	0	1.62	0	5.63	3.80	2.21
Gomani	0.31	6.06	9.81	8.67	17.56	8.48
Nyasungwi	0.30	2.83	15.97	82.75	12.81	22.93
Mbundumali	0.61	1.78	33.33	53.16	26.60	23.10
Kachamba	2.74	4.78	71.67	–	64.08	35.82
MH108	0.31	2.05	13.36	99.64	26.04	28.28

study was obtained from Makoka and Chitedze Research Stations, where rogueing was practiced in order to keep seed disease-free.

The results from this study are presented in Table 2. During harvest (12–14 months after planting), varieties Chitembwere and Gomani showed CMVD symptoms in 10% of plants. Variety Kachamba had the highest rate of infestation, followed by the Malawi Hybrid 108 (MH 108). MH 108 is not commonly grown by farmers. Makoka and Chitedze which are about 1000 m above sea level, had the much lower rates of infestation than the lowland sites.

ESTIMATION OF YIELD LOSS AFTER CMVD INFECTION

In order to have an idea of loss in yield as a result of CMVD in varieties commonly grown by farmers, further trials were conducted at the same sites (Tables 3 and 4). Variety Chitembwere had the lowest yield losses

Table 3. Mean yield of different varieties from cuttings free of CMVD (A) and affected by CMVD (B)

	Locality											
	Makoka		Chitedze		Chitala		Mkondezi		Baka		Mean	
Variety	A	B	A	B	A	B	A	B	A	B	A	B
Mbundumali	8515	1683	12217	3667	6234	1669	13897	1649	77222	31805	23617	8095
Kachamba	12064	287	10425	137	5764	1876	10503	1032	62139	50278	20179	10722
Chitembwere	5911	1331	7418	4653	5594	1234	15341	2948	81222	48611	23097	11755
Gomani	7768	12	6744	670	3308	175	16687	550	45916	25945	16088	5470
Mean	8569	829	9201	2207	5225	1239	14107	1545	66625	39160	20745	8996
S.E. ±	361.8		470.6		510.6		799.4		2820.1			
Sig. level	***		***		***		***					

S.E. for M x V±

Makoka	723.6
Chitedze	941.6
Chitala	733.4
Mkondezi	1598.6
Baka	5640.3

Table 4. Mean % yield loss in plots planted with CMVD cuttings, based on yield per hectare (A) and per plot (B)

Variety	Locality											
	Makoka		Chitedze		Chitala		Mkondezi		Baka		Mean	
	A	B	A	B	A	B	A	B	A	B	A	B
Mbundumali	80	80	71	60	69	57	86	81	52	47	72	65
Kachamba	98	91	99	72	66	75	91	90	34	39	78	73
Chitembwere	77	70	32	33	78	51	78	57	38	22	61	47
Gomani	99	99	83	69	91	80	97	93	53	42	85	77
S. E. ±	4.0	5.4	9.9	15.4	8.6	9.5	3.8	7.8	6.3	7.6		
L. S. D. (5%)	12.7	17.2					12.1	24.9				

in all sites, whereas Gomani and Kachamba had the highest losses. These trials demonstrate that, provided they are acceptable, some local varieties could be used in an IPM programme to limit the effects of the CMVD. More varieties need to be screened in order to identify those that are potentially useful for such a control programme.

MONITORING OF *BEMISIA* SPP. IN DIFFERENT CROPS

The aim of this study was to establish the host plant species range for *Bemisia* spp., with a view to identifying the crop plants which could be intercropped in order to reduce the adverse effects of the pest on particular priority crops. Yellow traps were used to monitor the movement of adult whiteflies in cotton, peanuts, sweet potato, vegetable (rape, drumhead and chinese cabbage), tomato and cassava. Eggs and nymphs on the leaves of these crop plants were also counted directly.

The highest number of *Bemisia* spp. adults were caught on traps placed in cassava, followed by tomato and cotton. Traps placed among chinese cabbage, drumhead cabbage and peanuts caught the lowest number of adult whiteflies (Fig. 2). Counts of eggs and nymphs also indicated that cassava is the preferred host plant (Table 5). No vegetables were included in these counts, however.

BEMISIA COMPLEX

It has generally been assumed that *Bemisia tabaci* is the predominant species found on cassava in Africa. In Kenya it is considered to transmit CMV and is found in relatively large numbers compared to other species such as *B. afer*. However, in Malawi studies in progress suggest

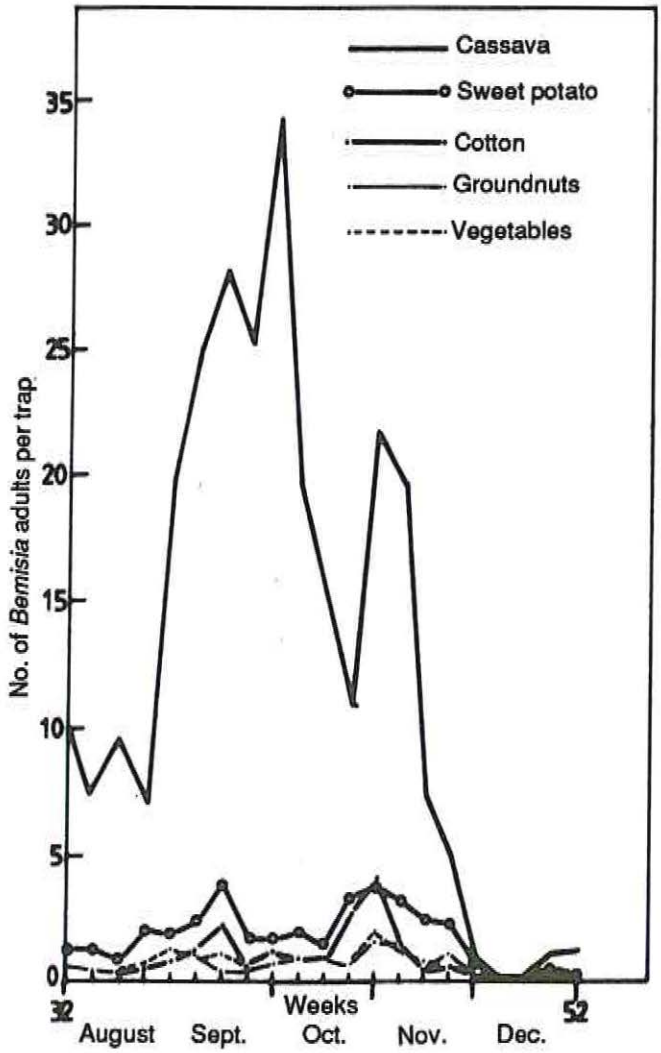


Figure 2. Mean weekly *Bemisia* catches in yellow traps in different crops 12 August–31 December 1986.

Table 5. Mean number of eggs and nymphs on leaf discs taken from different crops

Crop	Eggs	Nymphs
Cassava	2.24	2.47
Tomato	0.97	0.39
Sweet potato	0.94	0.08
Tobacco	0.96	0.00
Cotton	1.38	0.39

that *B. afer* is more predominant on cassava than *B. tabaci*. More studies need to be conducted to determine the species distribution on the other crops in Malawi. Inoculation work also needs to be carried out to determine whether both *B. tabaci* and *B. afer* transmit CMV.

NATURAL ENEMIES OF *BEMISIA*

Parasitoids

Although it is widespread elsewhere in Africa, *Eratmocerus mundus* has so far not been found in Malawi. The *Eratmocerus* parasites found in Malawi have not yet been identified to species (CIE identifications). *Encarsia transvena* (sublutea) has been identified in samples sent to CIE, but *Encarsia deserti*, which was introduced into Malawi in 1986, has not yet been recovered. The *Encarsia* specimens collected in Malawi include males, which is rare in many parts of the world. Parasitism were low, there was some indication of density-dependent activity. A clearer picture of the impact of parasitoids on the whitefly should emerge when data from other sites are analysed.

Predators

A number of predators have been observed during national surveys and during scouting at research stations. Studies aimed at quantifying their effect on the *Bemisia* populations are in progress in a number of sites. These predators include coccinellids such as *Scymnus* spp., which has been found to feed on adults and scales of *Bemisia* throughout the season. A neuropteran, belonging to the family Coniopterygidae, and *Semidalis* spp. were also found to be widespread in the country. It is generally more prominent where densities of *Bemisia* are relatively high. Many scales tend to be ruptured due to the predators' feeding activity. It is suspected that a combination of the action of predators and parasitoids may be responsible for the whitefly control effects observed at Chancellor College. However, more work is needed in this area.

Fungal Diseases

During these studies fungi were seen to have attacked and killed a number of scales, but their impact has not yet been quantified. A few infected scales were sent to the Commonwealth Institute of Mycology (CIM) for identification. The report from the CIM suggested that fungi were responsible for the mortality of the whitefly scales.

FUTURE WORK

The studies during this phase have mainly been exploratory and have raised a number of questions which need to be clarified. The whitefly species complex and its distribution need to be established, in order to understand the differences of CMVD incidence in high and low altitude areas. What may appear to be host plant resistance may just reflect differences in population levels of *B. afer* and *B. tabaci* to transmit cassava mosaic virus. Understanding the whitefly complex is different from that found in other countries in Africa. Local cassava germplasm needs to be collected so that it can be evaluated for resistance and tolerance to both the CMVD and the whitefly. The host plant range of *Bemisia* in Malawi also needs to be established. Parasitoids, predators and fungi need to be evaluated in terms of their impact on whitefly populations. The preliminary observations also indicated that the rate of disease transmission was faster in low altitude than in high altitude areas. The basis for this observation needs to be understood.

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Integrated Pest Management in Senegal

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Integrated Pest Management in Senegal

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INTRODUCTION

Pearl millet is the major food crop of the West African Sahelian Zone that includes eight countries: Cape Verde, Senegal, the Gambia, Mauritania, Mali, Burkina Faso, Niger and Chad. Recently Guinea Bissau became the nineth member state.

In these areas, a wide range of pests, including birds, insects, pathogenic fungi and weeds are known to attack pearl millet. Of these, insect pests are the most injurious (Risbec, 1950; Appert, 1957; Ndoye, 1979a). Although millet insect pests occur throughout the Sahel, their specific incidence varies according to rainfall and cropping patterns. The deficit rainfall conditions in the Sahel led to increased pest incidence. Crop intensification and diversification and growing of off-season crops led also to increased pest incidence.

ECONOMIC IMPORTANCE OF PEARL MILLET PESTS

There is consensus that insect pests on the whole are injurious to pearl millet. However, specific studies have rarely been conducted on the relative economic importance of the different species or complexes. Studies in Senegal and Nigeria confirmed the importance of the spike worm (Bos, 1983) and stem borers (Harris, 1962; Ndoye, 1977). Recently, the blister beetle damage has been observed in Gambia, Mali, Mauritania and Eastern Senegal, but no accurate evaluation has been made. The hairy caterpillar and spike worms have assumed major pest status since the recent drought and are serious problems of pearl millet.

MAJOR PESTS OF PEARL MILLET

The millet stalk-borer completes 2–3 generations during the cropping season. The adults emerge about 1 month after the first rain. The source of infestation is larvae that pass the dry reason in diapause in stalks and left in the field after harvest and panels made from plant stems (Gahukar, 1983). *Acigona* larvae devour the leaf whorl by penetrating the main veins, tunnelling through the stems above the node level and feeding on the stem pith. Subsequent dessication of the central leaves

results in dead heart formation — the plant may develop axillary tillers but is non productive. Spike worms, *H. albipunctella* have become major pests since the 1972–74 drought in the Western African Sahel. Monitoring the *Raghuva* complex showed that *H. albipunctella* was the most destructive species in Senegal. This species is also widespread in all the Sahelian countries (Ndoye, 1979b).

Adults emerge 1 month after the first rains. There is only one generation per year. The young larvae perforate the glumes and devour the floret core which can be detected by the small whitish granular excreta. Mature larvae cut through the peduncles in a characteristic spiral. The extent of damage depends on synchrony of adult buildup with early heading, density of larval population, plant-pest interactions and damage. Flight activity normally occurs in August and September (Ndoye, 1988).

At the end of the cropping season, the mature larvae descend to pupate in the soil where the pupae enter diapause and remain inactive during the off-season. In clay soils the pupae lie close to the soil surface (5–15 cm deep), but in sandy soils they are buried deeper (15–30 cm). *Many other insect species that damage pearl millet in the Sahel are considered secondary pests. Control methods developed combine traditional and new technical methods.* Although millet is a subsistence crop in this ecological zone, farmers cannot accept high cost control methods for this crop.

HIGHLIGHTS OF CONTROL METHODS

Cultural Techniques

Research was undertaken to understand the potential role of different methods to control insect pests of pearl millet. Cultural techniques such as fires, tillage, partial burning, bagging and destroying stalks, planting time, crop maintenance and fertilizer can be easily developed by the farmers. Lighting fires around the millet fields at night is a common practice in the rural Sahel to reduce blister beetle populations.

Deep ploughing either at the end of the rainy season or before planting the next crop can reduce large populations of diapausing *Amsacta* and *Heliocheilus* pupae in the soil (Vercambre, 1978).

Partially burying pearl millet stalks immediately after harvest destroys 61–84% of the larvae and 98–100% of the pupae of *Acigona*. The rate is 66–78% for larvae and 99% for pupae when stalks are put in plastic bags (Gahukar *et al.*, 1986). These two methods, carried out

carefully do not affect the quality of stalks used for making walls, fences or roofs. Cutting the stalks left in the fields to provide fodder for animals decreased residual populations of the pest.

As far as planting time is concerned, late planting of short duration varieties reduces spike worm infestation. Lastly, nitrogen fertilization significantly increases plant height and improves plant growth; heads are more vigorous and less prone to spike worm attack (Gahukar, 1983); however, 50 kg/ha N and 30 kg/ha P did not significantly influence infestation, in fact stem borer incidence was higher and caused stems to break before harvest (Gahukar, 1983). These cultural techniques do not cost too much and are easy to handle by the farmers.

Insecticides

One or two applications at flowering stage of endosulfan 700 mg/ha a.i. can effectively control spike worms (Vercambre, 1978). Similarly, one application of thuricide (*Bacillus thuringiensis*) successfully checked infestation and larval population of *Heliocheilus* (Gahukar *et al.*, 1986). Problems related to insecticide treatments such as low economic returns, application techniques for tall traditional varieties, and residues in grains and stalks were discussed (Ndoye *et al.*, 1986).

Biological Control Methods

Several studies on varietal resistance have been conducted in the Sahelian Zone, but without artificial infestation, because appropriate methods for mass rearing millet pests have not yet been developed. Little data is available on the spike worm, the stemborer and the blister beetle complex. Certain genotypes seem to be capable of minimizing pest damage. Implementing methods using natural enemies (predators, parasites and pathogens) was carried out. Risbec (1950) has listed the natural enemies of most millet pests. Recently, about 20 auxillary parasites of *Heliocheilus* have been identified for different development stages. Among these, *Bracon hebetor* (Braconidae) and *Litomastix bambeyi* (Encyrtidae) appear to be of major importance with parasitizations of up to 95% for larvae and 48% for eggs (Bhatnagar, 1984). Their activity has a significant effect only at the end of the cropping season, specially in dry years.

Tables 1, 2 and 3 show the natural enemies identified on *Heliocheilus*, *Acigona* and *Amsactor*, respectively. The biology of *Bracon hebetor* and *Litomastix bambeyi* and their subsequent use as biological control agents has been studied.

Table 1. Predators, parasites, and pathogens observed on the eggs, larvae and pupae of *Raghuva albipunctella* in the Sahel

Order	Family	Species of insects/pathogen	Stage attacked
Predators			
Hemiptera	Anthocoridae	*Orius* spp.	egg, larva
	Pentatomidae	*Glypsus conspicuw* Westw	larva
	Reduviidae	*Ectomocoris fenesratus* Flug.	larva
		Katanga etiennei Schoutedon	larva
Coleoptera	Carabidae	*Chalaenius boisduvalii* Dejean	larva
		C. dusaultii Dufour	larva
		Pheropsophus spp. nr. *lafertei* Arrow	larva
Hymenoptera	Eumenidae	unidentified	larva
	Formicidae	unidentified	larva, pupa
	Vespidae	*Polistes* spp.	larva
	Chrysopidae	*Chrysopa* spp.	larva
Parasites			
Hymenoptera	Bethylidae	*Goniozus* spp.	larva
	Braconidae	*Apanteles* spp. (*ultor* group)	larva
		Bracon hebetor Say	larva
		Bracon spp.	larva
		Cardiochiles sp.	larva
	Chalcididae	unidentified	larva
	Encyrtidae	*Litromastix* sp.	egg
	Ichneumonidae	*Hardromanus* sp.	pupa
	Trichogrammatidae	*Trichogrammatoidea* sp.	egg
Diptera	Bombyliidae	*Thyridanthrax* sp. nr. *kappa* Bowden	pupa
Nematode	Tachinidae	*Goniophthalmus halli* Mes	larva
	Meramithidae	*Hexamermis* sp.	larva
Pathogens			
Fungi	Fungi Imperfecti	*Aspergillus flavus* Link	larva
		Aspergillus sp. (*Ochraceus* group)	larva
Bacterium		unidentified	larva

Table 2. Predators, parasites and pathogens observed on eggs, larvae and pupae of *Acigona ignefusalis* in the Sahel

Order	Family	Species of insects/pathogens	Stage attacked
Predators			
Arachnida		*Pyemotes ventricesus* Newf.	larva
Parasites			
Diptera	Chloropidae	*Ceratopogon risbeci* Seguy	larva
		Epimadiza sp.	pupa
	Phoridae	*Aphiochaeta* sp.	larva
Hymenoptera	Bethylidae	*Goniozus precerae* Risb.	larva
	Braconidae	*Apanteles sesamiae* Cam.	larva
		Euvipio rufa Szepl.	larva
		E. fascialis Szepl.	larva
		Glyptomorpha sp.	pupa
		Rhaconotus soudanensis Wilkn.	larva
	Chalcididae	*Hyporchalcidia soudanensis* Stef.	pupa
	Encyrtidae	*Euzkadia* spp. (*Integralis* Merc.)	larva
	Eulophidae	*Pediobius furvus* Gah.	pupa
		Tetrastichus atriclavus Wtrst.	pupa
	Ichneumonidae	*Chasmias* sp.	pupa
		Dentichasmias busseolae Hein	pupa
		Syzeuctus spp.	larva
	Scelionidae	*Platytelenomus hylas* Nixon	egg
Pathogen			
Fungus		*Metarrhizium anisopliae* (Mert.) Sorok	larva

Table 3. Parasites, predators and pathogens on larva and eggs of *A. moloneyi* in Senegal

Species of insect/pathogen	Stage attacked
1. Parasites	
Diptera	
Phoridae	
Megaselia spp. (Loew)	larva
Megaselia scalaris	larva
Tachinidae	
Carcelia spp.	larva
Chetogena spp.	larva
Exorista spp.	larva
Palexorista quadizonula Thompson	larva
Palexorista sp.	
Hymenoptera Ichneumonidae	
Charops tegularis Szepl.	larva
Nematode - Mermithidae	
Hexamermis sp.	larva
2. Predators	
Formicidae (unidentified)	egg
Calosoma senegalensis (Carabidae)	larva
3. Pathogens	
Bacterium (unidentified)	larva

B. hebetor is a very active larval ectoparasite with a life cycle of 7.10 days. But its performance can be inhibited by hyperparasites like *Eurytoma* sp. (Pteromalidae) and *Pediobus* spp. (Eulophidae) from Niger (Guevremont, 1983). *B. hebetor* survives on *Ephestia* sp., a pest of stored millet grain, during the dry season. Studies led to a specific way of using this parasite by implementing a simple rearing method in the granaries and transferring it to pearl millet fields in August–September. This method of encouraging the parasite is very easy to handle by farmers.

Litomastia is a polyembryonic egg parasite that remains in diapause until the first rain when it emerges from prepupal host larvae in the soil. Up to 800 parasites have been collected from a single larva. Two other parasites play a very important role in the biological control of *H.*

albipunctella. Among these parasites, *B. hebetor* is the only one we used in the biological approach to control insect pests at farmer's level.

Most of the parasites but *B. hebetor* are less active during the dry season when their insect hosts are in diapause and climatic conditions break the parasite-pest synchrony. Although local natural enemies of certain pests contribute greatly to reducing the insect host populations, they are not capable of controlling the pests. The introduction of well selected exotic species would be more useful. Exchange of East and West African material is planned, following indications from Mohyuddin and Greathead (1970).

IPM FOR MILLET

Integrated pest management combines various practical, efficient and economical methods to maintain pest levels below the economic threshold. In the case of millet crop, the methods tested include use of improved cultivars, apparently pests and diseases tolerant, release of parasites (*B. hebetor*) and only one application at early heading of an insecticide known to be safe for auxilliary fauna (endosulfan). Detailed studies on the biology and ecology of pests and their parasites, and on the different control methods, are required to determine a global strategy against the pearl millet pest complex (Ndoye *et al.*, 1984).

FUTURE PLAN

The analysis of the prevailing situation has led Sahelian countries to choose a global integrated pest management plan against pests of all food crops. The results from the CILSS integrated pests management project in the Sahelian countries would be useful in formulating a preliminary strategy, but this approach raised certain problems. In depth studies on economic thresholds and on the bioecology of the major pest are essential. In order to achieve this, pest surveys and studies on the dynamics of the major pests, particularly stem borers, spike worms and hairy caterpillar, should be advanced further.

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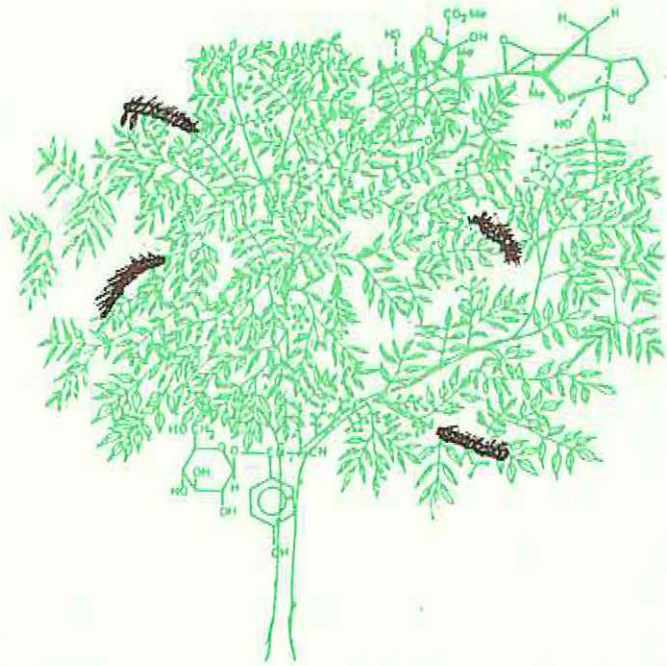


Observations and Recommendations

Observations and Recommendations

PHEROMONES

Pheromones have the potential to be used to monitor insect pest populations and to control pests by mass trapping or by confusing their mating behaviour with great speciality. However, todate there are only a few cases of successful control by pheromones. Among the problems limiting their use in biocontrol are:

1. While they are available for monitoring they must be combined with other control agents.
2. Research involved in their identification is very sophisticated and expensive.
3. Although useful, their specificity is daunting because of the number of different sets of pheromones that will be necessary to isolate and synthesize for different species and sometimes for the same species in different conditions.
4. They have to be dispensed in special ways to ensure steady release rates and protection from UV light and aerial oxidation.
5. Successful utilization of pheromones is contingent on intensive knowledge of the behaviour and ecology of the target pest in relation to meteorological conditions, vegetation types, cropping systems and so on.

BACTERIA, VIRUSES AND OTHER DISEASES AND PREDATORS OF INSECT/WEED PESTS

B. t.

B.t. is one of the longest used biological control agents, and over the past 40 years it has been used successfully under a variety of conditions for a number of insects. However, it is being marketed commercially with mixed results partly because its persistence is low and hence the need for repeated applications and partly because of relative specificities of different strains. Slow-release coated preparations have been more effective. However, there are concerns about the possibility of resistence to *B. t.* developing from its widespread and potentially "careless" application, and it should be seen as one control option rather than an exclusive one.

There is, therefore, need for:

1. Screening of new potential pathogens particularly local strains of *B. t.* and other organisms.
2. Standard protocols for detection of these organisms and bioassay of their efficacy/toxicity.

3. The development of better formulations of candidate pathogens and of "cottage industries" for the production of these pathogens based on simple fermentation technology.
4. Research on socio-economic aspects of pathogen use by farmers (possibilities of community participation in production, formulation and use, safety measures, cost-benefit analysis, etc.).
5. Research on *B. t.* strains or synthetic derivatives with greater persistence using biotechnology techniques.
6. Research in classical biocontrol linked to the behavioural ecology of the target pests and based on the deployment of natural enemies of the pests that are able to sustain themselves in the ecosystem (and do not require frequent application).
7. Development of effective application technologies and identification of appropriate stages of application to maximize impact of the intervention.
8. Research on herbicidal bacteria and fungi and associated phytotoxins for biocontrol of weeds.

NATURAL PRODUCTS AS BIOPESTICIDES

There are two broad categories of natural plant products used in the control of insect pests:

(i) Toxic preparations such as pyrethrum, rotenoids, limonoids, etc.
(ii) Behaviour controlling chemicals (antifeedants; repellents, inhibitors, etc.) such as asarones, ajugarins, etc.

Preparations based on such plant products have been used for millenia; however, few have received detailed scientific examination until recently. Azadirachtin (an antifeedant and anti-hormone) from neem, known in India 4000 years ago, has only now been synthesized.

Many are only known locally to farmers and a handful of medicinal plant specialists. They have the attraction of being natural products, locally available, and potentially being able to be produced by local cottage industries and used by farmers themselves. However there are many problems still to be overcome. The best known, such as pyrethrin, azadirachtin and rotenone, are broad-spectrum compounds that affect pests and beneficial species indiscriminately. The latter two have poor "knock-down" properties and may need to be compounded with other agents to be acceptable to farmers.

While pyrethrum is now widely marketed commercially, it is not yet clear if others will have a commercial future as cost-effective replacements for chemical pesticides. The major problems are:

1. Preparations are unstable and are broken down by heat, humidity and UV light. They may be effective only in uneconomically large doses. There is a need for more systematic development research on proven

safe and effective plant agents, such as rotenoids in the areas of enrichment, formulation and shelf-life improvement.

2. Applications are often undertaken without adequate research on most effective ways of application.
3. Effective plants are not available in sufficient quantities.
4. Screening of new plants based on community knowledge is generally unsystematic and requires careful selection of information and better methodology. Standard, replicable bioassays to determine effectiveness based on scientifically sound information is necessary.
5. Are antifeedants effective in the field? They tend to be species-specific and require high dose rates, so are expensive. Moreover, insects apparently adapt to them very quickly. Screening should be broadened to include other insect activities such as oviposition, growth and reproduction. Neuropeptides and muscle contractors are known in the literature and offer new research areas.
6. It is clear that most researchers limit screening of plant products to one or two bioactivities. For greater cost-effectiveness, research needs to be widened to include antifungal, nematicidal and herbicidal activities.
7. Research on the development of plant-based pesticides is often carried out by scientists representing a single discipline (chemistry or biology). There is a clear need for multi-disciplinary teams of chemists, behaviourists, ecologists, toxicologists, social scientists etc. to assess comprehensively both effectiveness and cost-effectiveness of natural products in pest control.
8. Potential hazards (health or ecological) of pesticidal plants and their natural products are ill-defined. There is need for understanding effects of those compounds on non-target pests and in the development of better guidelines for safety in their local preparation and use and in the introduction of exotic species (for propagation) in new ecosystems. A knowledge of the mechanism of action of active natural products would also be useful in assessing their potential hazards.

SYSTEMS DESIGN

The systems approach employed by the IITA Biological Control Programme goes beyond Integrated Pest Management to include:

- plant host phenology and physiology
- 1st trophic level — ecosystem effects on yield formation of agricultural crops
- 2nd trophic level — life cycle studies of pests and population dynamics
- 3rd trophic level — natural enemies population dynamics and epidemiology
- ecosystem context — agroecosystem, intercropping efforts, pest behaviour in absence of crops (bush sources of re-infestation)

- temperature-driven processes — solar radiation, soil nitrogen, organic matter, moisture, etc.
- Social and economic factors.

A computer model is then built, using laboratory parameters, which is then validated by field studies and used as a research tool to identify pest control ecological options, not only what is innovative but what is needed.

There is need for better collaborative efforts to establish uniform methodology, food sampling methods for population dynamics, better definitions of "noxiousness" and damage thresholds.

GENERAL ISSUES

1. *Intellectual Property Rights*

In many countries no patents on natural products are allowed. In Africa, appropriate legal devices must be evolved to encourage innovation, application and commercialization of natural products.

2. *Biosafety*

This is becoming increasingly important and an Africa-wide regulatory mechanism is needed. In some countries there is very little control, in others excessive caution limiting use of proven, safe preparations.

3. *Resources for Research and Development*

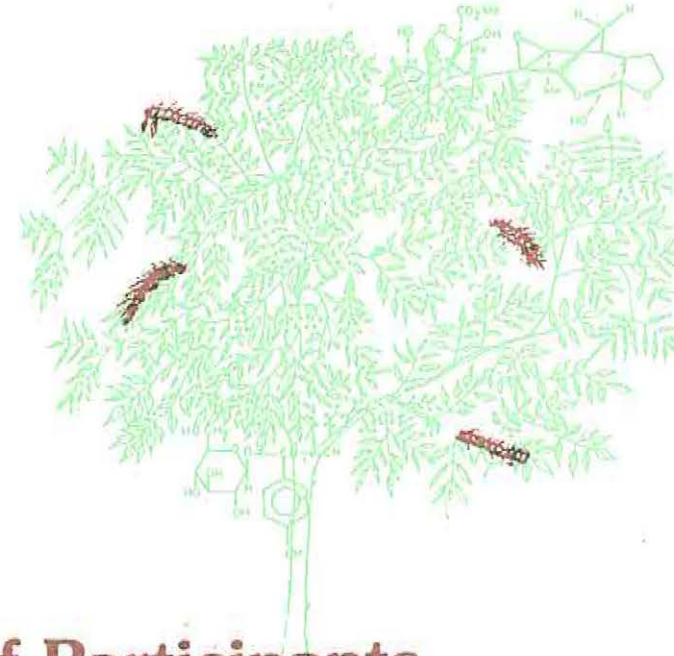
This fruitful area is hampered by lack of adequate funding and trained manpower. The United States based PSTC supports innovative research, but not surveys or local field trials for socio-economic data collection. The IITA/BCP budget has been cut in half. More understanding of the importance of the area, the potential benefits to be derived, and the research resources needed, needs to be stressed to donors and recipient governments.

4. *Networking and Collaboration*

An important theme that emerged from the workshop is the need to pool scattered efforts in the region together through exchange of information, collaborative research and provision of specialized services and training. A network of all institutions engaged in biopesticides R&D would promote this. The proposed Network would also address the following needs:

(a) training in different bioassays and in more specialized areas such as formulation technology and simple cottage-level fermentation or isolation procedures.

(b) a regional spectroscopic centre for natural products housed in an institution such as the ICIPE with capacity to maintain and operate different spectrometers. Perhaps the UNDP/IAEA could be approached to assist in setting of such a centre.



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