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STRENGTHENING OF PESTICIDE DEVELOPMENT CENTRE

DP/IND/89/128

INDIA

Technical report: Findings and recommendations*

Prepared for the Government of India
by the United Nations Industrial Development Organization,
acting as executing agency for the United Nations Development Programme

Based on the work of B.P.S. Khamby
consultant in Botanical Pesticides

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United Nations Industrial Development Organization
Vienna

* This document has not been edited.

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CONTENTS

1. EXPLANATORY NOTED

IPFT: Institute of Pesticide Formulation Technology

IARI: Indian Agricultural Research Institute

RES: Rothamsted Experimental Station (Institute of Arable Crops Research in England)

HSEB: Haryana State Electricity Board

RENPAP: Regional Network on Pesticides for Asia and Pacific

II. ABSTRACT

Title : **Botanical pesticides**

No. : **IND/89/128/11-62**

Duration : **2 x 1 month**

I.1 Objectives:

To advise on the establishment of a project at IPFT aimed at producing formulations based on pesticides of botanical origin. In this, the first of two planned visits, a primary set of targets (see Introduction) was defined.

I.2 Conclusion

The objectives of the Institute are realistic and can be achieved but an essential first step is the identification of a suitable species of plant for exploitation, and an understanding of the chemistry of the active compound(s). Success will be dependent on a multidisciplinary approach involving biologists to provide bioassay results and chemists to identify compounds. It should concentrate on lipophilic insecticidal compounds.

Since not all the necessary facilities and know-how are available within IPFT, collaboration with external Institutions is highly recommended (already initiated with IARI and RES). Contacts with a herbarium should follow.

Although the Analytical and Formulation groups at IPFT are well equipped, the Entomology group requires substantial upgrading both in facilities and safety requirements (Annexure 3). Training of staff is also of equal importance. If the bioassay facilities are created at a level commensurate with that of the Analytical and Formulation Section, then the Institute would indeed be the Centre of Excellence within the RENPAP countries. The Institute could then provide national and international standards and protocols in most areas of agricultural technology.

The heavy investment in sophisticated equipment should be matched by planning for future upgrading and replacement.

I achieved most of the targets defined for the first phase, but initiation of practical work was incomplete because of delays in obtaining equipment (See Annex 3) and training of staff.

III. BACKGROUND

This section starts from the basis of the contract as defined and therefore, without consideration of any economic or social factors either in isolation or in comparison, to the commercially available synthetic pesticides.

A number of reviews on plants as sources of natural pesticides indicate their potential. For example:

1. M Grainge and S Ahmed (1988).
   Handbook of plants with pest-control properties. New York, Wiley.

2. J T Arnason, B J R Philogene and P Morand (Eds) (1989).
   Insecticides of plant origin, ACS Symposium Series **387.** American Chemical Society, Washington DC.

3. H G Cutler (Ed), (1988), **Biologically active natural products: Potential in agriculture,** ACS Symposium Series **380.** American Chemical Society, Washington, DC.

4. H Schmutter and K R Ascher, (1985), **Natural pesticides from the neem tree and other tropical plants,** Proceedings of the 2nd Int. Neem Conf, Rouisscholtz-hausen, Germany, Germany, GTH.

Botanical pesticides such as pyrethrins, rotenone, nicotine, veratrum alkaloids and quassia were widely used before the advent of synthetic pesticides. With the onset of resistance, and public concern over synthetic pesticides, attention has once again turned to natural products (especially plant-derived) as pesticides. The sophisticated methods of formulation developed for synthetic products have been applied only to a limited extent to the natural pesticides already in use. The opportunity offered by the lack of exploitation of this aspect is a good reason for the exercise defined by my contract.

However, present knowledge does not identify additional obvious species or compound for immediate exploitation; for lack of information on

1) activity against a range of pests including selectivity towards beneficial species.

2) Stability in the field

3) acute and chronic toxicology against mammalian species

IV. INTRODUCTION

This report was in consultation with Bhupinder P S Khambay and deals with the establishment of a project aimed at the eventual production of commercial formulations from pesticidal plants of India (Annex 1). The work has been planned in two phases to correspond with two visits by the consultant. Targets set for the first phase were:

a) Assessment of existing facilities and expertise at IPFT

b) Advising how to select plants with pesticidal activity

c) Recommendations on formulation of protocols for bioassays and isolation of active principles.

d) Advice on the initiation of practical work

Duration of the first visit was one month. Major contacts including names of counterparts are listed in Annex 2.

During the second visit, emphasis will be on optimising the procedures along with advice on monitoring and assessment of field efficacy of selected formulations.

V. ACTIVITIES

A data base was created on the IPFT's in-house computer to record details of plants investigated which will be particularly useful in future to avoid duplication.

Much time was spent in discussions and practical demonstrations of the techniques and protocols. Unfortunately, during the first week a training course was being held at the Institute and access to equipment (e.g. rotary evaporator) was not possible. Furthermore, even the basic glassware had to be ordered externally and was delayed due to two public holidays in the second week. Even in my last week, only some of the glassware and chemicals had arrived.

Mr Bhateshwar and I visited University of Delhi for two days to explore the possibilities of collaboration, and were joined later by Mr Dhari at IARI. During the visit I gave a talk on research at RES, especially on botanical pesticides and also the proposed IARI and IPFT collaboration. The discussions culminated in a meeting with Dr S K Sinha, Director of IARI who endorsed the proposed collaboration. The next step was to proceed with the administrative procedure.

At the outset, the laboratory was poorly stocked with equipment and solvents. Thus, initiation of basic work on extraction of plants and bioassay procedures was delayed by several days. In addition several public days disrupted a whole week resulting in further delays in deliveries from external suppliers. Although several insect species had been examined in the past (Annex 4) only mosquito larvae (all three species) and the pulse beetle were currently available, because insects are reared either in single generations or seasonally due to lack of proper rearing facilities.

Preliminary investigations on alcoholic extracts at *Annona squamosa* and *Ipomea cornea* had been previously conducted, but without proper overall protocols. In the case of *Annona squamosa*, a great deal of work has been reported in the literature and activity attributed to polar alkaloids and more recently to some lipophilic compounds (acetogenins). However, previous work at IPFT was based only on ethanolic extracts. Therefore further work on *Annona squamosa* should only be carried out if high levels of activity can be detected in the lipophilic extracts (i.e. hexane and ethyl acetate extracts).

In the case of *Ipomea cornea* only a low level of activity was found against mosquito larvae. However, the anti-ovicidal activity reported in the literature may be of greater significance. An appropriate bioassay is being established.

Both *Annona squamosa* and *Ipomea cornea* have been extracted sequentially with ethyl acetate and 95% ethanol and subjected to preliminary bioassay.

Work has started on mosquito larvae bioassay to evaluate the effectiveness of lipophilic compounds in this test. In the absence of a standard, DDT was recommended. Initial work was aimed at determining the type and maximum limits of organic solvents concentrations. The shape of containers holding the larvae was also found to be critical especially when organic solvents were added. The present and future requirements of the bioassay needs are listed in Annex 4.

Further work on extracts was limited due to unavailability of any insect suitable for topical testing. Larvae of *Spodoptera litura* are expected to be available within the next two weeks.

VI. RECOMMENDATIONS

1. Formulation and practical realisation of the proposed collaboration with IARI and RES is crucial to the overall success of the project.

2. A chemist should be appointed within two years to coordinate all aspects of the project.

3. Close interaction between Analytical and the Biological Sciences groups at IPFT is essential.

4. Establish contact with an authority (e.g. herbarium) to facilitate in the classification and location of plants. This arrangement should be particularly useful when carrying out taxonomic searches.

5. Initially, only those plants should be considered which have established insecticidal activity associated with lipophilic compounds (see SECTION III for references). General screening to identify new interesting plants should be a secondary priority.

6. Bioassays should be standardised with reference materials (e.g. DDT, nicotine, rotenone).

7. Maintain regular cultures of several insect species. The choice of insects should be based on a range of orders and pests of economic importance.

8. Upgrade laboratories (Annex 3).

9. Training for Dr N R Bhateshwar, Project Coordinator (minimum 3 months) and Mr B C Mandal, (minimum six months) who will be mainly concerned with extraction and fractionation of active compound (RES has been tentatively proposed for training).

10. Work should be aimed at formulation of extract containing lipophilic insecticidal compounds. This would capitalise on the in-house expertise and also avoid problems of environmental pollution (through leaching in soil) of water soluble compounds such as leaching in soil, or inherent mammalia toxicity e.g. of alkaloids.

11. Extraction protocol should be based on sequential extraction with three solvents (e.g. hexane (or equivalent), ethyl acetate and 95% ethanol).

12. Separate parts of the plants should be investigated individually (e.g. flowers, bark, leaves etc.).

13. Initially the extraction and evaporation of solvents should be carried out below 35°C to avoid possible decomposition or rearrangement of active compounds. Once the structure is known then for large scale extractions conditions would be optimised.

14. Future work could include *Mammea longifolia*.

ANNEX 1

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANISATION
JOB DESCRIPTION
IND/89/128/1-62

Post Title	:	Consultant in botanical pesticides
Duration	:	2 mm
Date required	:	Oct/Nov 90 or early quarter of 1991
Duty station	:	Pesticide Development Centre (PDC) Udyog Vihar, Gurgaon 122 016, Haryana, India

Expert will stay in New Delhi and commute to PDC

Purpose of project: To develop locally available pesticidal natural products for local needs.

Duties: The consultant will be required to advice and assist scientists associated with the Pesticide Development Centre on:

i) Selection of plants having pesticidal activity

ii) Extraction and isolation of active ingredients

iii) Characterization and identification of active components

iv) Lab screening of toxicological properties

v) Formulation of botanical pesticides

vi) Development of Quality Control procedures

vii) Field evaluation of botanical pesticides

At the end of his mission, he is expected to submit a report on his work carried out at PDC, his findings and recommendations.

ANNEX 2

Contacts

Dr Kawal Dhari - National Project Coordinator

Dr N R Bhateshwar - Senior counterpart at IPFT

Dr P K Ramdas
Dr S Y Pandey
Dr Y P Ramdev
— associate counterparts at IPFT

Mr A Singh - Dy. Secretary to Ministry of Chemicals & Fertilizers

Dr S P Dhua - Regional Coordinator, RENPAP

Dr S K Sinha - Director for IARI

Dr S Ghai
Dr B S Parmar
Dr D B Saxena
Dr C Devakumar
Dr G Kulshrestha
Dr D S Singh
Dr R P Singh
— IARI, New Delhi

Prof S K Mukerjee - Ex IARI

Dr B S Kapoor - University of Delhi

Dr R N Khanna - University of Delhi

Mr K K Sharma - Chairman, Northern Minerals Ltd

Dr S Gupta - Dhanuka Chemicals

ANNEX 3

ASSESSMENT OF INSECT REARING AND LABORATORY FACILITIES

A. Available

1. Insect rearing rooms - Two
2. Laboratory for bioassay - one
3. Air conditioners - two (one is old and out of order, not repairable)
4. Hot air blowers - four
5. Trays, bowls, jars etc (adequate)
6. Potters tower and a microapplicator

B. Additional requirements

1. Insect rearing room - one (10 x 12 ft)
2. Air conditioners - three
3. Hot air blowers - four
4. Insect rearing cages - six
5. Rotary vacuum evaporator - one
6. Columns for flash chromatography - range
7. Vacuum pumps - one for rotary evaporator and one for high vacuum
8. Solvent fumehood and solvent storage cabinet

C. Arrangements for continuous power supply

Interruptions in HSEB power supply is a regular occurrence in Haryana. Although during day time backup is provided by generators, there is no backup system for out of working hours. Disruptions in power supply to the rearing rooms would results in loss of cultures. It is therefore proposed to have a separate automatic power generation with a capacity to run 3-4 air conditioners at a time.

ANNEX 4

INSECT CULTURES FOR BIOASSAYS

1. *Aedes aegypti*, 2) *Anopheles stephens*, 3) *Culex fatigans**

Type of assay intended

i) Continuous exposure in water
ii) Anti ovipositional activity

4. *Spodoptera litura***

Type of assay intended

i) Topical application
ii) Treated leaf feeding

5. *Diacrisia obliqua***

Type of assay intended

i) Topical application
ii) Treated leaf feeding

6. *Triboleum castanium**

Type of assay intended

i) Direct spray by Potter tower
ii) Dry film exposure

7. *Callosobruchus chinensis**

Type of assay intended

i) Topical application
ii) Treated grains

8. *Musca domestica* - **culture to be initiated**

Type of assay intended

i) Direct spray by Potter tower
ii) Topical application
iii) Dry film exposure

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* Cultures are regularly maintained
** At present reared in single stages or seasonally

UNIDO COMMENTS

The report deals with the various requirements for the development of botanical pesticides. Being a field of multi-faceted activities from discovery to commercialization of botanicals, the IPFT can play only a defined role within the vast area of developing pesticides. This means, as the report indicates, that close collaboration is needed with a number of organizations in India and outside India. The author who comes from a world renowned institute for the development of botanicals, has offered to take a few fellows for training. This should be taken up by IPFT at the earliest and also initiate concrete action to collaborate with the Indian institutions.

While the report gives advice carrying out work on basic R&D in botanicals, we prefer this to be given to universities or R&D laboratories of CSIR and only concentrate on development work.

The report also provides establishing reliable laboratory testing facilities at a reasonable cost and this should be coordinated with the Indian Agricultural Research Institute which has already built up a good working relations with IPFT.