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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 Saulo de Jesus Soria,
Consultant in pesticide bio-efficacy

Backstopping officer: B. Sugavanam, Chemical Industries Branch

United Nations Industrial Development Organization
Vienna

* This document has not been edited.

ABSTRACT

Consultancy activities on insect rearing techniques and to develop pesticide efficacy testing facilities were carried out for two months at Pesticide Development Centre (PDC), Udyog Vihar, Haryana, India, as a part of UNIDO Government of India Joint project IND/89/128/A/01/37, within a programme for the strengthening of PDC Phase II, 1991. The objective of mission was to advise on developing pesticide testing facilities in the country. It was concluded that mentioned project is being conduced normally, but for having complete execution needs support for building insectary and bioassay laboratory.

CONTENTS

List of Annexes

INTRODUCTION

This report summarizes the work carried out during a period of 6 weeks at Pesticide Development Centre (PDC), Udyog Vihar, Gurgaon - 122 016, Haryana, India. The writer was recruited by UNIDO to assist the project on strengthening of Pesticide Development Centre Phase II with emphasis on development of pesticide efficacy testing facilities in the country. The duties were set out in job description No. Code IND/89/128/A/01/37, as follows:

." The expert is expected to advise and assist scientists of PDC on a consolidated plan to improve existing facilities of insect rearing for testing pesticidal formulations for agricultural, horticultural and public health outlets:

. Additional equipments with specifications if necessary, needed to carry on efficient, reliable and reproductible screening experiments in tropical conditions with pesticidal formulations developed at PDC and other laboratories:

. To board methodology for interpretation of results and proper documentation for logical comparisons of results with standards."

As usual, the expert will have to submit a report on his findings, work carried out and recommendations.

After brieffing in Vienna, the writer left for New Delhi, arriving there on 10th May 1991. He left New Delhi on 29th June, 1991. The original objectives were fully attained.

This report should be read in conjunction with DP/IND/80/037, March 1987, entitled "Technical Report: Review of Entomology Section facilities and staffing at Udyog Vihar (PDC) and recommendations for upgrading these, based on the work of Quinton A.Geering, Consultant on Crop Protection and Public Health, and in conjunction with DP/ID/SER,A/737, 9' December 1986, entitled "Technical Report: preparatory assistance for the establishment of Biological testing facilities, based on the work of Clive E. Price, Expert in biological evaluation of pesticide formulation.

RECOMMENDATIONS

For UNIDO and the Government of India :

1. To build up insectary and bioassay laboratories, with basic design features outlined in annexed drawing. drawing.

2. To promote training on insect mass rearing, bioassaying and other toxicological subjects for Bioscience staff. A list of laboratories is included as suggestion to choose the most convenient for every case.

3. Provide equipments as listed in enclosed annex: a jeep or pickup, a glasshouse with heating and cooling facilities, a desk microcomputer and other listed are necessary for the research programme.

4. Re-activate subscription of widely circulating scientific journals that bring toxicological information and subscribe PDC Library to some journals of other tropical countries. Some suggestions are given in annex.
For the Government of India:

5. To plan and implement programme of visits of Bio-science group to toxicological research laboratories, some suggestions included in annex.

CHAPTER I

OBSERVATIONS MADE ON PRESENT LABORATORY FACILITIES, FIELD TRIAL FACILITIES AND STAFF NEEDS.

With regard to this subject, I refer to Dr. Geering's 1987 Report, in all details.

At present, Dr. Geering's proposals were partially implemented. For example, Anopheles rearing room and bioassay facilities were implemented with individual air-conditioning unit, permitting continuous work, even in the extremely hot conditions registered during summer season in Haryana (reaches nearly 40° C at shade). It is widely known that insect rearing in aquatic ecosystems stand better warm temperatures, because water plays a thermoregulatory roll. Air born insects, on the other hand, would not support such extremes of temperature nor low relative humidity levels of under 25%. However, the present rearing facilities and laboratory testing facilities are meagre and inadequate to meet the day to-day research activities. For this reason, it is suggested to establish as a short term goal, the building of an insectary unit for airborn insect rearing and bioassaying. Particular design features are proposed to counterpart particular climatic conditions registered at Delhi and Haryana region. An architect will be able to adapt particular features into technical standards (see drawing Annex No.4).

If needed to maintain insect cultures, and provide a regular supply of insects for laboratory bioassay, then a new insectary unit will be essential. Insectary is proposed to be built in separate site, within the PDC territory, with associated glass-house, storage of rearing ingredients and field trial facilities. (Dr. Geering REPORT pg. 5) Storage of chemicals, however, is proposed in separate unit from insectary unit.

Particular architectural feature refers mainly to the need of insectary wall be protected from direct impact of lateral sunlight rays, prevalent during most part of the year, due to its 28° latitude North of PDC geographical location. Additional architectural feature may represent an additional cost in building budget, but will be largely compensated with fruitful

research results. Failure in taking into account protection of lateral walls from sun rays will turn them into infrared ray emissors that will heat the interior of insectary rooms, making impossible the rearing of insects during summer.

Current research activities are mainly restricted to

(i) breeding of mosquitoes and conducting bio-assays comparing Bacillus sphaericus and B. thuringensis var. israelensis formulations.

(ii) another important research project at Biosciences department of PDC is collection, identification, extraction and screening of different botanical plant species to find out the toxic principles as insecticides. The basic objective of the project is to find out the most active principle component in the easily growing plant species, isolation of the toxic component, its identification and characterisat

(iii) Field trials planning and preparations for next monsoon season are in progress.

CHAPTER II• LABORATORY INSECT REARING

Insect rearing activities at PDC, by the time the writer was visiting (May-June, 1991) near the peak of summer, was restricted to breeding mosquitoes i.e. Anophelesand Culex spp. A method to continuous rearing is well established, adults being able to intake blood meals from domestic pigeons and rabbi

Continuous rearing of terrestrial airborn insects has been handicarped in summer season because extreme unfavourable environmental conditions, mainly related to extremes registered for temperature (40 or more ^{o}C under shade) and very low relative humidity (25%), under which not even coackroaches are able to stand. The writer was informed that other insects, collected in the field, may be used for laboratory bioassay, earlier in season. Disadvantages of field collected populations are well known because they bring strong heterogeneity ingredien in the population. For this reason, a suggestion is registered to build up a new insectary and bioassay laboratory, to counteract these environmental limitations. Annex No. 4 refers to general architectonic features that will be necessary to be considered to obtain a fresher internal environment within the laboratories.

Due to the need at PDC of continuous effect of laboratory insects for tests, it is included here,
cheap methods of rearing coackroaches and house flies, as done in Brazil (St.paulo Biological Institute method). It is necessary to register here the existence at PDC library MRS. Carroln Smith; book on insect mass rearing, with alternative suggestion as to rearing methods of coackroaches, house flies as well as other insects.

Also another justificative for the proposal of new insectary refers to present location of Entomology section, immediately below the formulations Laboratory, with attendant risk of Contamination of insect cultures.

CHAPTER III. THE BIOLOGICAL EVALUATION OF PESTICIDES

Data on bioassessment of the effect of several doses and several formulations of bio-insecticides on mosquitoe Anopheles stephensi are being regularly taken at PDC laboratories. The subject that is being investigated refers to the assessment of two microbial insecticides Bacillus sphaericus (2362) and B. thuringiensis var. israelensis against mosquitoe 3rd instar larvae in standard mosquitoes bioassay trays. The method consists in submitting 50 larvae per tray, to several doses, or formulations= as the case may be, of the active ingredient, repeated three times. The method includes I control, repeated three times and II blank, repeated also three times. Tests are being carried out, but no analyse was made yet. It is suggested that probit-log polo programme (Leora software, Berkeley, California) to be used forquick and accurate analyse of data. In order to imput data for the programme, following sequence in the array of data is suggested:

Ist. column	2nd column	3rd column
dose of the product, or concentration	number of assayed insects per tray	number of insects dead per tray

If data inputs are in the programme in this order, the programme will bring information on percent values of killed insects, LD_{50} values, null hypothese pest values, fiducial limits for LD_{50} s for each array of data. Above mentioned programme is left by the writer for use at PDC, but he suggested that PDC could eventually buy its own copy. Buying the copy may bring some advantages to PDC since it may facilitate further contacts with the mentioned software enterprise and may protect PDC against eventual claims for its use in publications. Due to the fact that above mentioned research project is being carried out within internationally accepted standards, comments and criticisms in this stage of development turn out to be unnecessar

CHAPTER.IV.- FIELD SCREENING OF PESTICIDES

Field screening activity at PDC normally takes place in two seasons, i.e. kharif (monsoon) and rabi (winter) seasons. Field tests corresponding to kharif season were carried out on evaluation of different formulations of Butachlor, a rice herbicide, in rice crops at PDC. Preparations for the next monsoon season field trials with butachlor formulations have started. In the rabi season, the PDC Bioscience staff were engaged in the field screening of different formulations of Isoproturan, a selective herbicide against grass weeds in wheat crop and different formulations of Carboxin, a systemic fungicide against loose smut of wheat.

By the time the writer was present at PDC, May-June, neither of the two field screening activity periods were coincident for work. So consultancy work was carried out in the area of training, by having discussing seasions with Bio-science staff members at PDC. A handout on field screening subject was prepared and is included as an Annex No. 10.

In relation to field screening activities, some additional equipments as knap-sack motorized sprayers, and electrically powered motorbooms are included as items to be bought, with specifications in Annex No. 3 .

With reference to training activities in this production area, the main points that were discussed during round table meetings refer to the mechanism of analysis from a statistical standpoint. For this purpose it was prepared a handout to orderly introduce the subject in a stepwise manner in short chapters.

Another research project that is being carried out at PDC refers to bioassessment of botanical plant species for insecticidal activity. The method consists in testing alcohol extracts from seeds, roots against Dichrisia sp., for example and against mosquito larvae. According to selected target to be protected, two prospective methods could be proposed. For the case of leaf eating insects, where insecticidal action is desired, the killing active principle should be concentrated so that the product may be competitive with other insecticides available in the market. This consideration refers mainly to the prospective use of neem extracts against agricultural pests. Levels of bioefficacy in field tests may vary according to the type of use of the product. U.S. Department of agriculture may accept a 80% mortality threshhold as a criterion for recommending the product for agricultural use for airborn insects whereas 95% field mortality will be the threshhold limit for Environmental Protection Agency (EPA) standards, in the U.S.A.

In case of use of repellent botanical extracts by humans or domestic animals, then the assessment will be made under the standards of repellent products.

Additional equipment is suggested to be acquired to upgrade laboratory conditions at PDC (List in Annex3).

CHAPTER V.- TRAINING PROGRAMME

In relation to quality of scientific staff of PDC it could be observed that training overseas offered to three scientists of Bioscience section proved to be highly beneficial for the research programme. This statement is based on evidence of rising level in the quality of research standards in toxicological work with particular reference to field trials.

From what was observed during present mission, it was suggested to give the scientific staff an opportunity to receive training on laboratory insect rearing, bioassessment and other related toxicological subjects overseas. Some laboratories are suggested (Annex No. 9) but previous contacts should be made in order to ensure proper attention to the trainee.

CHAPTER VI CONCLUSIONS

1.- Programme for strengthening of pesticide Development Centre, Udyog Vihar, Haryana, India, through the joint project of UNIDO and PDC IND/89/128/A/01/37, is being implemented and executed within operative plan 1990-1994.

2.- Regarding insect rearing, it was observed that serious limitations exist as to adapt existing laboratory facilities to rear airborne and soilborne insects, due to external environmental extremes - temperature and relative humidity- registered in summer season. It is concluded that PDC needs a new insectary and bioassay laboratories facilities to counteract these limitations.

3.- Implementation of airborne and soilborne insect rearing facilities for bioassaying at pesticide development centre should be strengthened.

4.- Regarding biological evaluation of pesticides, it was implemented the use of polo-software programme to analyse bioassay data, with obvious advantages if compared to the manual system of analysis.

5.- It is concluded that collaborative project should be continued since research results are showing the positive aspect of this interaction.

A C K N O W L E D G E M E N T S

Sincere thanks are due to

Mr. Sat Pal, and all those at UNDP, New Delhi and at PDC, Udyog Vihar for generous help and assistance.

I thank in particular to Dr. Kawal Dhari, General Manager of PDC, for extending facilities required to accomplish the programme of work, Dr. N.R. Bhateshwar, Dr. Y.P. Ramdev and Mr. S.P. Yadav for help and continuous encouragement during the course of work.

Annex No.1 Counterpart and Scientific Staff at Biological Sciences Department of PDC

• **Dr. N.R. Bhateshwar** Manager Product Development	:	M.Sc. in Agricultural Entomology, Ph.D. in Entomology (insect. toxicology), Specialise in pesticide activity ar pesticide residues. Presently working as He of the Department of Biological Sciences.
• **Dr. Y.P. Ramdev** Entomologist	:	M.Sc. in Entomology (ins physiology), Ph.D in Entomology. Specialised in insect activity and mode of action of insecticides.
• **Mr. S.P. Yadav** Junior Entomologist	:	M.Sc. in Agricultural Entomology.
• **Mr. J.P. Degra** Field Supervisor	:	B.Sc. (Agriculture), Post Graduate Diploma in Plant Protection
• **Mr. Yashwant Singh** Junior Scientific Assistant	:	B.Sc. (Agriculture)
• **Mr. B.C. Mandal** Junior Scientific Assistant	:	M.Sc. in Zoology with specialisation in Entomology

Annex No. 2. Contacts made with people on subjects related to Mission

List of people met and contacted : at Indian Agricultural and Research Institute, New Delhi-12.

1. Dr. J.S. Gill, Division of Nematology

2. Dr. Swaraj Ghari, Division of Entomology.

3. Dr. K.N. Mehrotra, Division of Entomology.

4. Dr. Ram Nath, Plant Pathologist, N.B. of Plant Genetic Resources

5. Mr. C.L.Kanaujia, Head, Library Services

Contacts made by letter with :

6. Leora Software Ltd.,
Leora Software
1119, Shattuck Avenue,
Berkeley, California 94707, U.S.A.
Subject : Buying Polo Probit-log programme for bioassay analyse of date.

7. Dr. J.G. Krishna, Professor
Department of Entomology
College of Agriculture,
Rajendranagar,
Hyderabad-500130, India. Telephone 245161 Ext. 233
Subject : Insect Toxicological Research in India

8. Dr. R.K. Dutta, Technical Director
Rhone-Poulenc Agrochemicals (India) Ltd.,
Rhone Poulenc House,
S.K. Ahire Marg,
Worli, Bombay-400 025, India.
Tel. 493 1031, Fax 494 4277, Telex 011 76576
Subject : Insect toxicological Research in India

9. The Computer Sciences Librarian
Pacific Southwest Forest and Range Experiment Station
P.O. Box 245,
Berkeley, California 94701, U.S.A.
Subject : Requests micro-computer programme for probit bioassay analyse of data

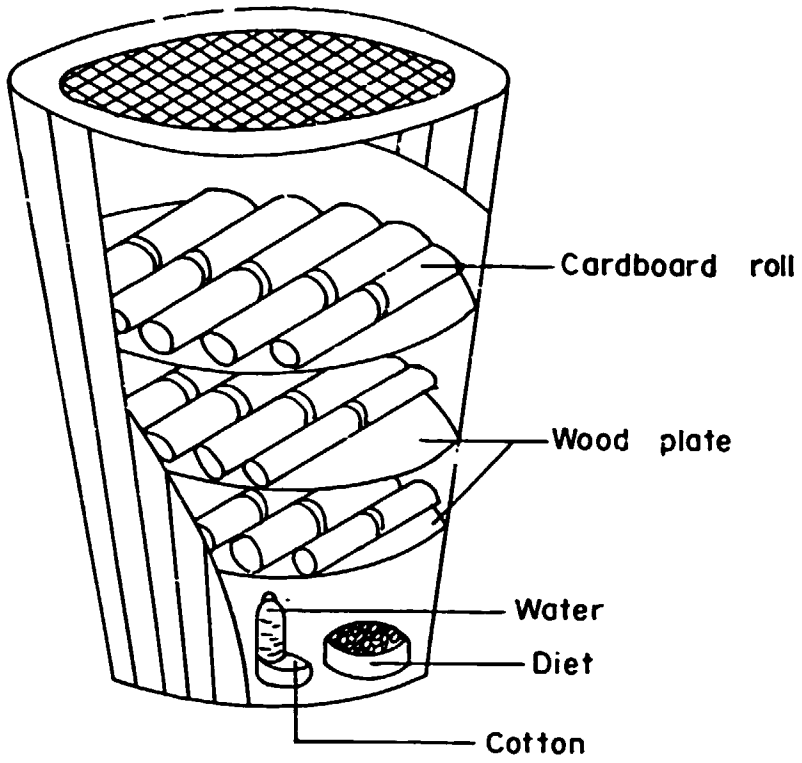
Annex No. 3.

Equipment to be provided by UNDP: Major items with cost and technical specifications.

Item	Amount	Estimated Cost($)
1. Air conditioning units	10	5000.00
2. Termostatically regulated incubators	03	4500.00
3. Desk personnal Computer (PC)	01	2000.00
4. Recording Thermohygrographs	06	6000.00
5. Power operated field sprayer	01	2000.00
6. Motorised costal knapsack sprayer	03	3000.00
7. Pickup or jeep-type vehicle	01	30000.00
8. Climatized plant growth chamber	01	5000.00
9. Rechafgeable 12V battery	02	100.00
10. Transformers to recharge 12V battery	01	100.00
11. Black light lamps for insectary (unit)	10	20.00
12. Electric vacuum pump	02	1000.00
13. CO_2 cylinder (unit 20 Kg.)	02	100.00
14. LeOra POLO probit-Log software analyzing programme	01	100.00
15. Stereo-microscope, 10-80X lens, with light equipment.	02	2000.00
16. Field digital manual counter (unit)	10	100.00
17. Field digital electrical recorder	02	400.00
18. Talgreen funnels, to sample soil insect battery with 6 funnels	01	200.00
19. Berlesse funnels, to sample stored product insects.	05	100.00
20. Malaise traps, for air-borne insect	02	50.00
21. Johnnson and Taylor suction trap, for air-born insects.	02	30000.00
22. Entomological nets	50	100.00
23. Photographic camera, lens 1:2.8 or 2:4 for film 135, professional.	01	400.00
24. Close-up lens, equipped with zoom devise	01	150.00
25. Time-lapse photography devise, for serial photography	01	200.00

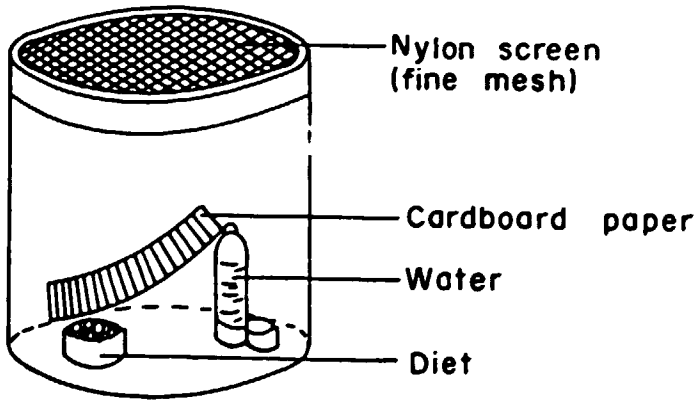
26.	Wide angle lens, "bull's eye"	01	150.00
27.	Teleobjective lens	01	150.00
28.	Flash with rechargeable battery	01	200.00
29.	Tripod, professional	01	100.00
30.	Portable case	01	50.00
31.	Peet-grady chamber, 6'x6'x6' (sold by Chemical Specialities Manufacturer Association (CSMA), specifications in Soap and Sanitary Chemical Blue Book, England).	02	200.00
32.	Greenhous with benches and coolers (Unit)	01	50,000.00

Annex No. 4
List of Figures



Big containner for adults and nymphs bigger than 1 cm

Figure 1



Small containner for nymphs smaller than 1 cm

Figure 2

Male: Posterior border of 9th sternite smooth or slightly indentated.

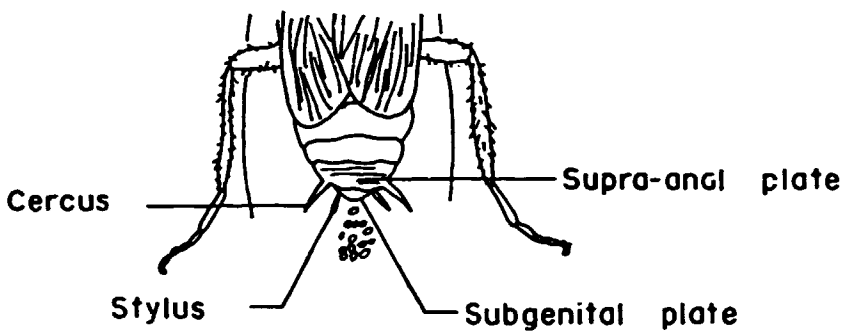


Figure 3

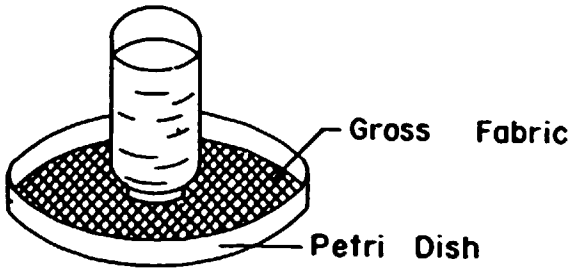
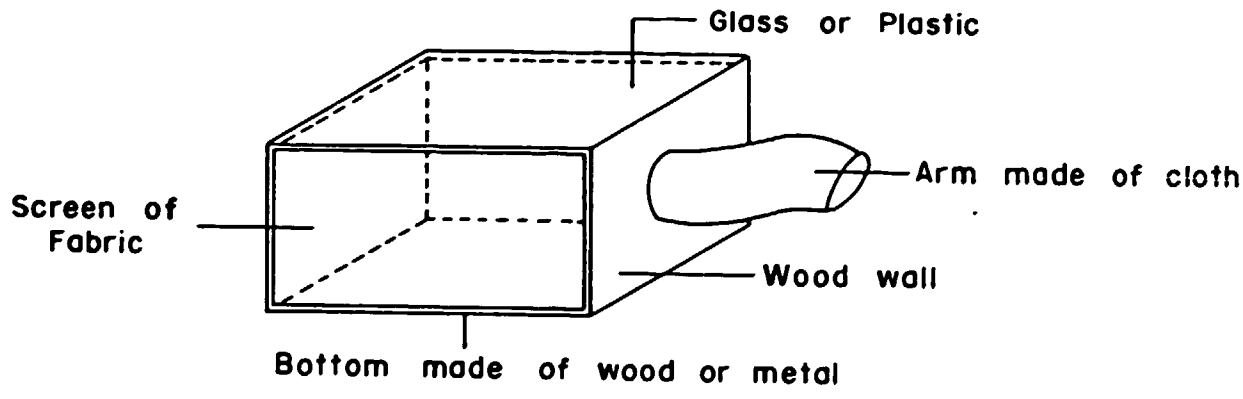


Figure 4



Capacity: Ex. 50 x 50 x 50 cm = 10000 Pupae

Figure 5

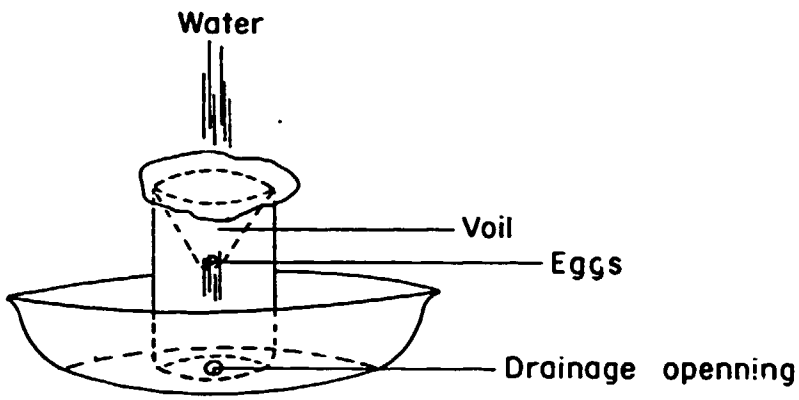


Figure 6

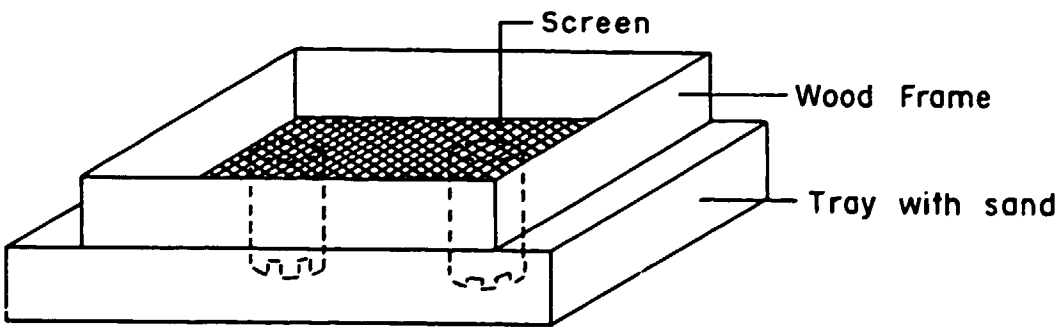
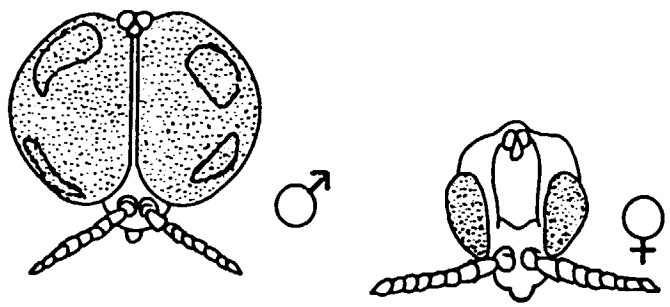


Figure 7

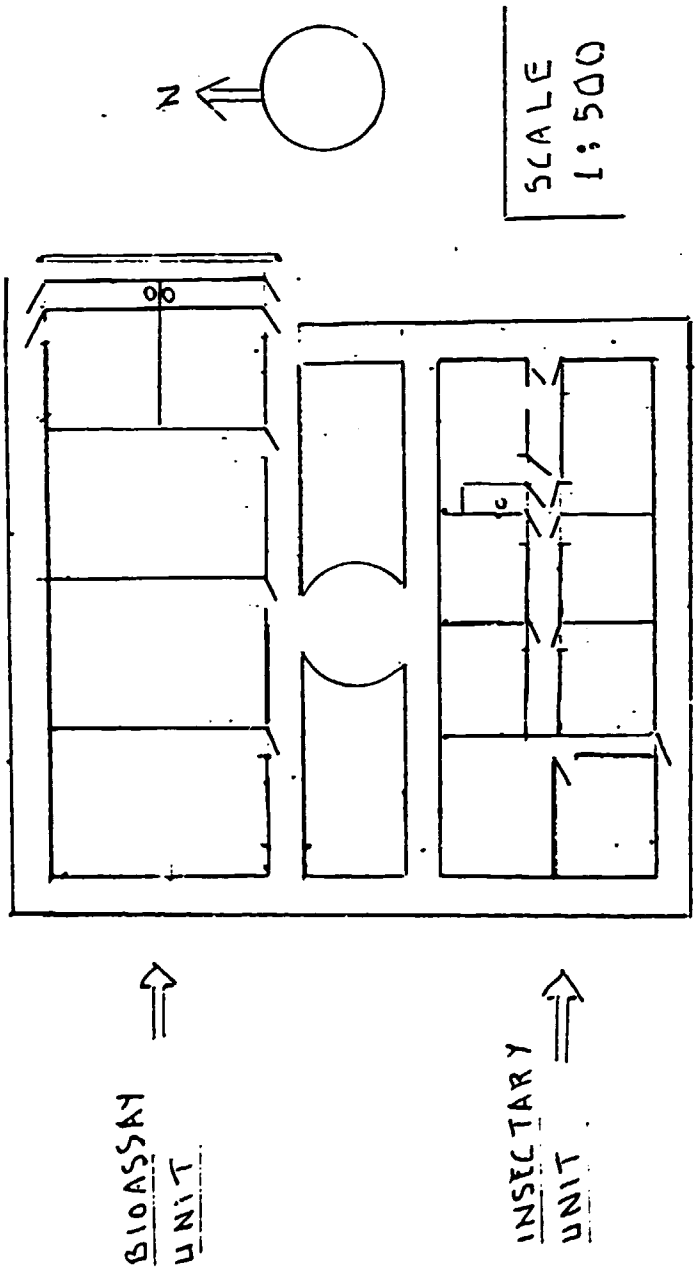
Sex diferentiation by the eyes (Ribeiro, 1951).



Adults: Males - Holoptic (eyes close to each other)
Females - Dicoptic (eyes separated)

Figure 8

FIGURE 9.- HORIZONTAL CUT OF INSECTARY AND BIOASSAY UNITS.



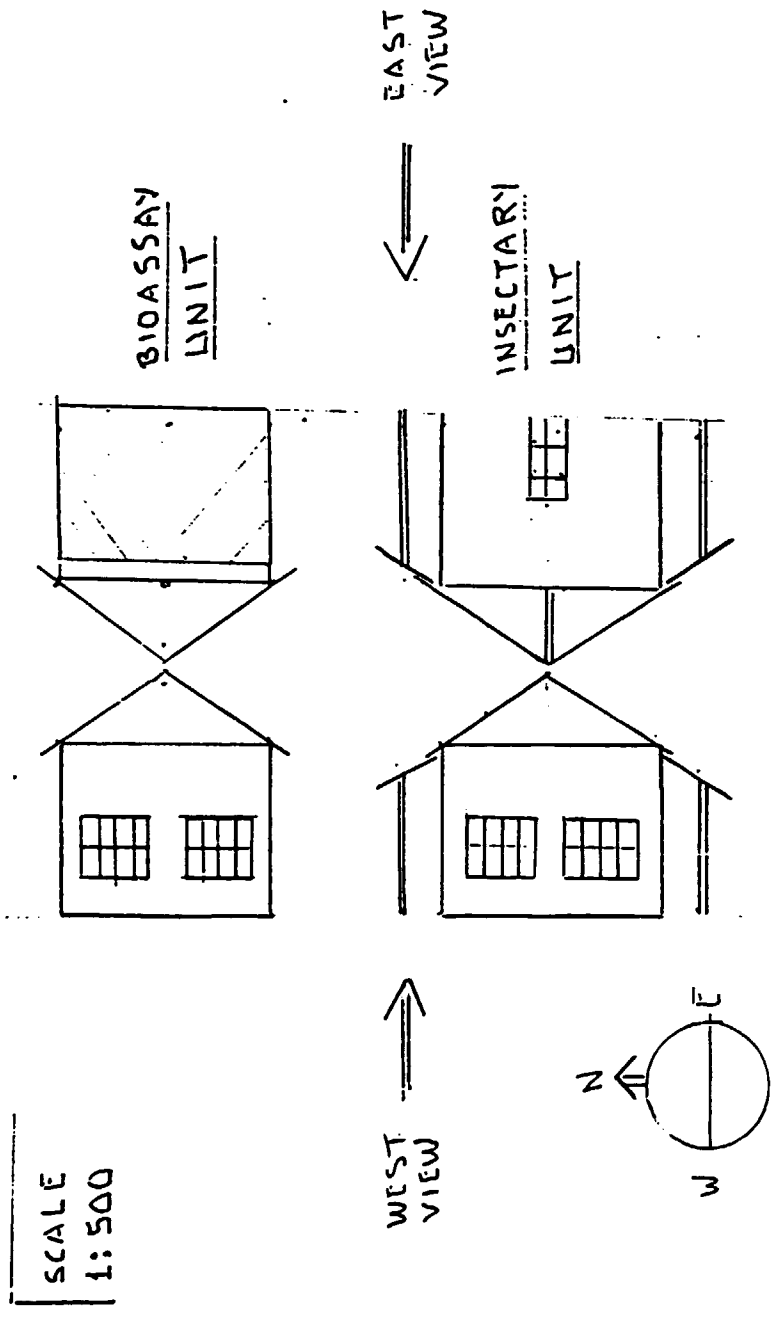


FIGURE 10 - WEST AND EAST SIDE VIEWS OF INSECTARY AND BIOASSAY UNITS.

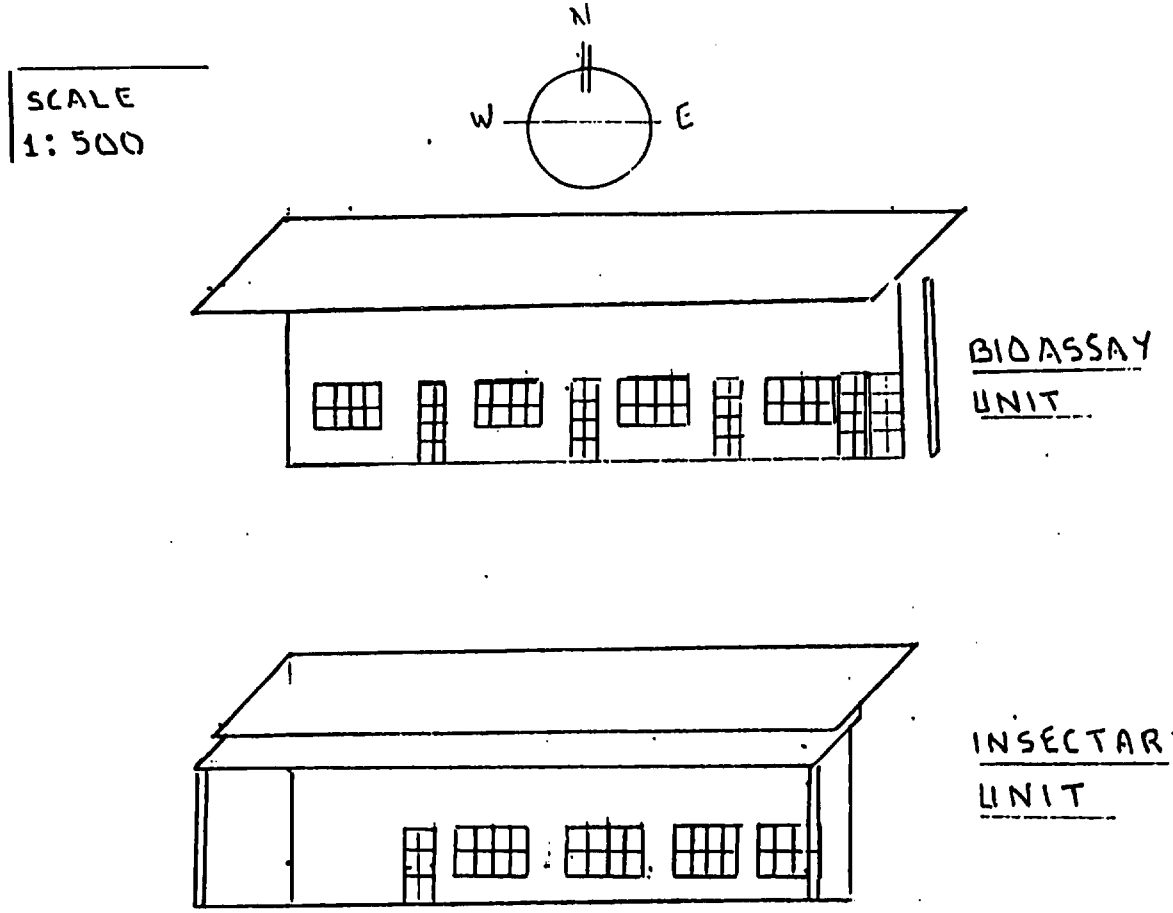


FIGURE 11.- SOUTH SIDE VIEW OF INSECTARY AND BIOASSAY UNITS, RESPECTIVELY.

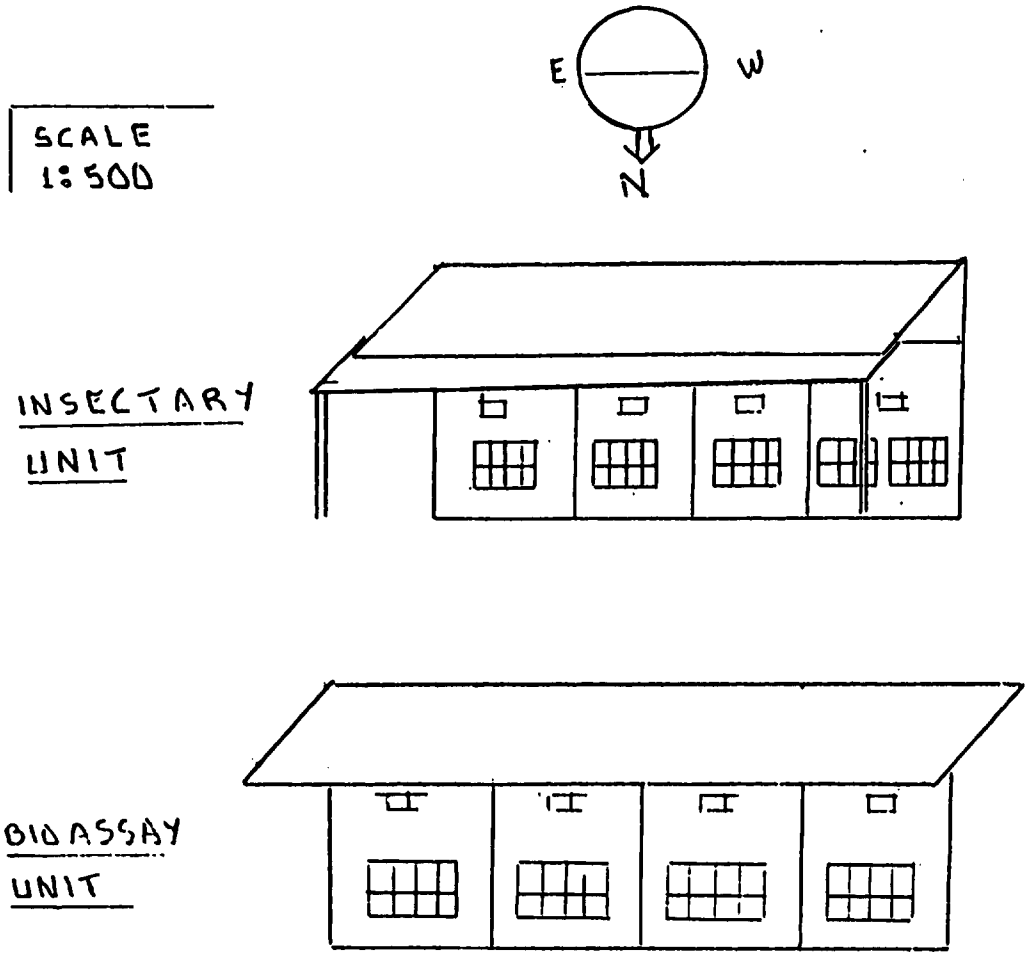


FIGURE 12.- NORTH SIDE VIEW OF INSECTARY AND BIOASSAY UNITS.

Annex No. 5.

ITINERARY AND WORK UNDERTAKEN

05. May 1991. Left Bento Goncalves (Brazil) to Vienna.

07. May. pre-mission brieffing in Vienna.

09. May. Departure Vienna 10:20 hrs. to N. Delhi

10. May. Arrived New Delhi 2.30 hrs. AM Report to UNDP.
New Delhi -local brieffing with Mr. Sat Pal, UNDP - Officer and Dr. Kawal Dhari,General Manager of PDC.

13. May. I am reported to work at PDC, Udyog Vihar, Gurgaon Haryana.

Significant portion of time was dedicated to consult members of Entomology Sector at PDC and preparing talks, report and recommendations.

In addition, the following visits were made:
Indian Agricultural Research Institute, New Delhi
Nomatology Department, Entomology Department, Genetins Deptt. and Library Services Deptt.

28. June. Technical debrieffing in New Delhi with Mr. Sat Pal and Dr. Kawal Dhari.

29. June, Leave New Delhi.

Following work was carried out :

1. Study and assessment of existing bioassay facilities, several discussions with staff.

2. Visits made, following scientists were contacted:
Dr. J.S. Gill, I.A.R.I. Nematology Department, subject discussed, nematological research in India:
Dr. Swaraj Ghai and Dr. K.N. Mehrotra, I.A.R.I. Entomology Deptt. subject discussed: Entomological research in India.

Mr. C.L. Kanaujia, Head of Library services of I.A.R.I. discussion on library facilities at I.A.RI. New Delhi.

3. Organised technical talks in round table meetings wtt Bio-Science staff. Following subjects were aboarded :

- Techniques of rearing some laboratory insects: examples with tropical insects.
- Laboratory bioassay assessment, analytical approach.
- Field screening of pesticides and bioefficacy assessment.
- Selected cases of economical entomology problems in tropical conditions.

Annex No. 6. Suggested books to be acquired :

1. Busvine, J.R.1971. A critical review of the techniques for testing insecticides. 2nd Edition. 345 P. Commonwealth Agric. Bur. Inst. Entomol., Dorset Press. Dorchester (England).

2. De-Bach, Paul, Ed. 1968. Biological Control of Insects and Weeds. Berkeley, University of California.

3. Finney, D.J. 1978. Statistical method in biological assay (3rd. ed.). London, Charles Griffin & Co. Ltd. 508 P.

4. Matsumura, F. 1975. Toxicology of Insecticides, New York, Plenum Press. 503 P.

5. Mc-Kenna, Conner. 1987. Pesticide Regulation Handbook Executive Enterprises Publ. Co. Inc. 480 P.

6. O'Brien, R.D. 1967. Insecticides : Action and Metabolism. New York, Academic Press. 332 P.

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8. Peterson, A. A Manual of Entomological Equipment and Methods. Vol.I-1934: Vol.2-1937

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10. Wadley, F.M. 1967. Experimental Statistics in Entomology. Washington, D.C. Graduate School Press. 133

11. Singh, P. and Morre, R.F. Handbook of insect rearing. Amsterdam, Elsevier, 1985,. 488 P. Vol.

12. Singh, P. Artificial diets for insects, mites and spiders. New York, IFI/Plenum, 1977. 594 P.

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CAMPBELL & MOULTON;Chemical Contnol of Insects, AAAS Nº 20:3-81. 1943.

DeBEER, E.J. 1945. The calculation of biological assay results by graphic methods. The all-or-none type of response. J. of Pharm. and Exp. Therapeutics 85:1-13.

DEWEY, J.E. 1958. Utility of bioassay in the determination of pesticide residues. J. Agr. & Food Chem. 6:274-281.

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Annex No.8. Suggested journals to be subscribed or reactivate subscrption

- Indian Journal of Entomology
- Indian Journal of Malariology
- Indian Journal of Agricultural Sciences
- Indian Management
- Dengue Newsletter
- Tropical Pest Management (London)
- Journal of Economic Entomology
- Annals of the Entomological Society of America
- Environmental Entomology
- American Entomologist (former Bulletin of ESA)
- Canadian Journal of Zoology
- Revista Turrialba (Costa Rica)
- Pesquisa Agropecuaria Brasileira (Brazil)
- Annais de Sociedade Entomologica do Brasil (Brazil)
- Annais da Sociedade Brasileira de Entomologia (Brazil)
- Annais de Sociedade Brasileira de Zoologia (Brazil)
- Anales de la Sociedad Argentina de Entomologia (Argentin
- Anales de la Sociedad peruana de Entomologia (Peru)
- Arquivos de Instituto Oswaldo Cruz (Brazil)
- Bragantia (Brazil)
- Arquivos de Escola Nacional de Agricultura ENA (Brazil)
- Annals de la Societe Entomologique Francaise (France)

Annex No. 9.

List of Departments, Universities and campuses where formal toxicological courses and research training are offered overseas.

Department	University	Campus	Senior Professor
Entomology	California	Berkeley	John Cassida
Entomology	California	Davies	Fumio Matsumura
Entomology	California	Riverside	Mir S. Mulha
Entomology	Wisconsin(USA)	Madison	P. Linchestein
Entomology	Cornell	Ithaca	J.E. Dewey
Entomology	Sao Paul (Brazil)	Piracicaba	G.C. Batista
Entomology	Oswaleo Cruz Institute (Brazil)	Rw De Janeiro	Joseph Joubere

It is also suggested that bioscience research staff be allowed to visit following laboratories :

- Pesticide Toxicology Laboratory
  The University of Agricultural Sciences
  GKVK Campus
  Bangalore-560 065, India.
  Telephones : 367553 & 365206

- Pesticide Toxicology Laboratory
  Fredrick Institute of Plant Protection and Toxicology
  Padappai-601 301
  Chingleput Distt.
  Tamil Nadu, India,
  Telex No.041-7547

The objective of the visit will be to strengthen scientific interchange.

ANNEX Nº. 10.

FIELD SCREENING OF PESTICIDES

CONTENTS :

Some general principles :

Population distribution and behaviour of insects have to be considered in planning field experiments of insecticides and other chemicals.

All standardized experimental designs used in agricultural and biological research are based on the assumption of normal distribution of insects in the field. Field practice demonstrate however, that this type of distribution may not always occur.

Insect population distribution may show sometimes skewed distribution. Poision distribution, binominal and negative binominal distribution are frequent in field conditions. Therefore, special analyse may be applied for each case.

In experiments where plants are host elements for insects or pathogens it is assumed that they are normally distributed, therefore standard experimental design may be applied to entomological research.

Sampling and size of sample

Systematic net sampling will work better with flying or jumping insects, which may be caught with a net. Pre-establish fixed number of sweeps, before starting a row may render representative data.

In case of more sedentary insects, local counting in host plant may be recommendable.

In case of tree-orchards, knock-down check may prove to be practical. Pyrethrum or 12% BHC can be used for the case of tropical orchards. In case of colonial insects, mortality should be based in the killing of queens.

Insect countings should be done wright away. Some insects will suffer organic decomposition within short time.

In case of knock down, insects may be preserved in alcohol for further countings.

Size of plot, is determined according to the representativity of the size of sample. In potatos, for example, a plot of 75' x 18' 6 rows, would be representative. But a plot of 30' x 50' would be better for lettuce. Lettuce occupies less square area than potatoes.

In case of moving insects, is suggested to use rectangular plots. Latin square is not advised because of vicious neighbouring effect.

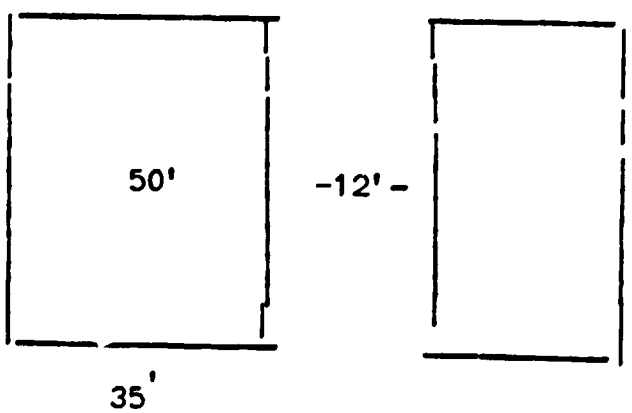
Plot distribution in the field should take into account insect behaviour in a case-by-case basis. It may be permitted establishment of rules for avoiding agglomeration of treatments in one side, giving regional influence.

In case of soil insects, linear rows may become perfectly good plots.

Establishment of experiment in the field have to follow some precautions : Dealing with moving insects, it is necessary to increase the number of repetitions, or, to increase the size (surface) ofthe plot in order to obtain more accurate measuring of populations.

Great vegetal barriers are effective to prevent leaf hoppers (CICADELLIDAE, DELPHACIDAE, ETC) moving back to the plots In case of aphids, they move more hazardously, but a couple of rows of cereal grains or corn will prevent aphids and leaf hoppers moving from plot to plot.

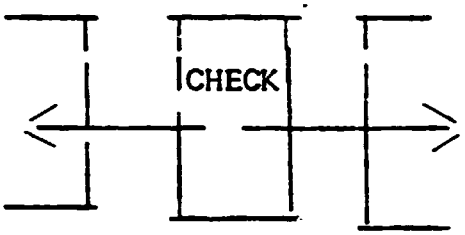
For Field screening plots mearuring 50' :: 35' with a border of 12' between plots have been used successfully.



Use of check plots and borders :

An insecticide experiment should be careful in planning the use of checks, several situation may occur:

1) Insect population of check plot may dilute into bordering plots.



2) Contrary effect may also happen in the case of chemicals having repellent effect on insects. For example, brodeaux mixture has repellent action congregatting insect population in check.

3) In case of suspicion of a behavioural effect of the solvents used to dilute the chemical under test, additional check, where some solvent is applied, should be taken into account.

4) There will occur cases also in which mechanical effect of application technology may induce increased yields. Then, additional check, where application technology is used without the chemical, should be considered.

5) In case of an insecticide screening experiment, in which fungicides are also applied, it is recommended to consider two checks: one without insecticide, and one without fungicide.

6) It is important to consider the lasting effect of the insecticides example; thrichlorphon lasts 1 week, methyl parathion as much as 2 weeks, endosulphan more than 1 month.

7) In all cases, border lines with same or other crops will always prevent drift effect of chemicals and of moving insects.

Big scale experiments at farm level have to be carefully planned because of high cost in services and time spent in its conduction. Again, size of plots will depend on the insect and the crops.

DOSE - MORTALITY AND THE TIME MORTALITY EFFECTS

The hypothese tested refers to the effect of an active ingredient on a population of insects or plants, etc.

In case of testing several doses of a compound the approachs in the analyses will be programmed; LD 50 analysis, or analysis of variance (ANOVA).

In case of time - mortality effects, the hypotise tested refers to the effect of time on mortality. Time interval may be divided in geometric or log scale of 1, 2, 4, 8 at HRS after application. should help to have meaningful data analyse and conclusions.

In case of screening of several compounds, experimental design should be planned to carry out analyse of variance (ANOVA) plus any other discriminative test, DMS, Tuckey, Duncan, etc.

Selection of experimental design.

Take into account insect population aspects and host plant population aspects.

Completely Randomized block design :-

Rules : randomize plots within each block.

It would fit best for annual crops, field crops, and also perennial crops, fit best for all crops.

Mathematical model: $X_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$

advantages:

Can programme blocks separately, so, highly adjustable for field conditions:

able to separate clearly

effect of the chemicals

effect of the block (horizontal variation, soil, drift, others).

Ideal, 4 replicates. Increase in the number of replicates will not result in increase of efficiency of experiment: Experiment should occupy the least area possible.

Concept of size of plot: exists an ideal for each crop, please consult agronomist for each case.

Border effect important. Prevent border effect by perviewing additional crops line only to avoid continuity from the plot to the next.

In case of flying or jumping insects, consider border effect, high volume sprays, low volume sprays, mist sprays, aerosols, etc., border effect should be very carefully watched out. Some drift will always occur.

Mathematical model for a two way classification :

$$X_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$$

where,

i = 1...a,

j = 1...b.

Where μ represents the overall mean, the α_i stand for treatment effect, and β_j for block (replication, horizontal) effects. It is assumed that :

1. The mathematical form implies that treatment and horizontal effect are additive.
2. These are independent random variables, normally distributed with mean 0 and variance V^2. They represent the extent to which the data depart from the additive model because of experimental error.

Completely randomized plot design

Appropriate for green house, glass house, or place where will occur little or none horizontal variation.

Mathematical model : $X_{ij} = \mu + \alpha_i - \varepsilon_{ij}$, where $\begin{matrix} i = 1...a \\ j = 1...n \end{matrix}$

Fixed effect model : The effects of treatments, measured by parameters are regarded as fixed, but unknown quantities to be estimated.

Advantages: simple and handy.

Disadvantages : Unappropriate for field condition.

PREPARING FORMULATIONS SPRAY SOLUTIONS. SUSPENSIONS FOR FIELD USE.

Dealing with insecticides, some precaution measures to prevent toxicological hazards against human beings, and other non target life forms should be taken into account in a case-by-case basis:

1. read always the manufacturer label of the product, with technical instructions of use;

2. make choice of formulation, accordingly with your needs, to put several types of formulations within a new experiment may result more difficult to work (will need differenciated equipment in resources and time spent).

3. in case of Organo-phosphate insecticides is advisable not to make dilutions much time before using them, because most of them suffer hydrolysis:

4. in case of water suspensions of wettable powder, advisable the use of insecticide stickers or surfactants. Both are necessary for the better dispersion and adhesion of the insecticide, fungicide or herbicide:

5. Even in case of emulsifiable concentrates, stickers and surfactants are suggested to help dispersion of the active ingredient:

6. Use of stickers depend on the type of leaf of the crop under experimentation. The dose of stickers will vary between 2-4 onces/100 gallon in case of non-water repellent leaves and the dose will double (4-8 onces/100 gallon) for the water repellent type of leaves i.e. cabbage, onions etc.

7. In case of plants with high water repellent properties such as cabbage, onions, etc. imulsifiable concentrate formulations will adhere better, will be necessary to review the dose of active ingredient, since dose depend on formulation to some extent. Some amount of insecticides can be more phytotoxic in emulsive solution than in wettable powder, flobo, granules or microencapsulats:

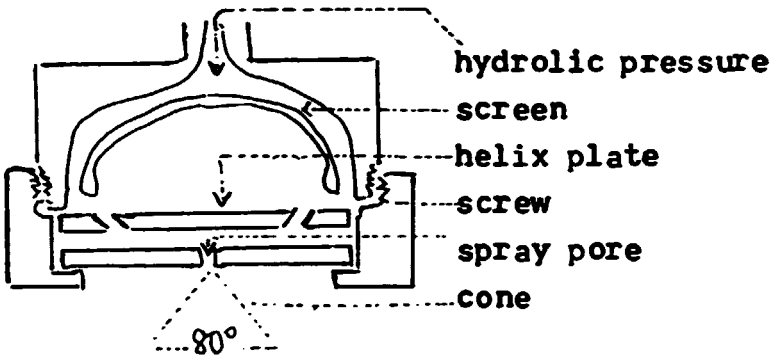
8. For the case of plants highly sensitive to chemicals (carica sp.) there are special horticultural oils for avoiding phytotoxicity. Agrochemical dealers have list of them usually well refined fraction of petroleum refinery or vegetal oils prove to be safe and are used for every particular case.

APPLICATION TECHNOLOGY OF PESTICIDES

Preparing equipment for field use :

Nozzle basic components :

Figure = BASIC NOZZLE COMPONENTS



Through the manipulation of these components, output rates can be regulated. If all components are regulated, it can be obtained various size of droplets, various pressure, gallenage per acre, and angle of dispersion.

Calibrating spray equipment

If needed great adhession of chemical to the leaves, high volume spray application will be advisable : 100 gall./ac enough to wet down all foliage of an established host, at a pressure of about 100-200 pounds/ square inch.

In case of use of dry powder, 30 pounds of talc/acre, will be enough to distribute the chemical in the whole acre.

With regard to hydrolic pressure, 100-200 pounds/ squared inch work well. On the other hand, if much pressure (200-400 pounds/ inch^2) the material does not adhere well to leaves, tending to jump off the leaves in a ping-pong effect.

In case of low volume application, upto 50 gal./acre, it will be necessary to set up nozzle components to obtain hollow spray cone, and to do finner drops. Doing of finner drops can be obtained by increasing hydrolic pressure.

Selection of high or low volume application system will be made in a case by case analysis. For example, low volume application of less than 60 gall./acre, will not be effective for controlling aphids in potatos : On the other hand, same low volume application will turn out to be effective for leaf hoppers (Cicadellidae, Piesmatidae, : Homoptera) and leaf beatles.

In case of stomach venoms (B.thuringensis, B. sphaericus), arsenicals, etc.) high volume application advisable, covering all surfaces, from three angles. Add stickers and surfactants, that function like an insurance practice.

Effective doses obtained at laboratory, glass house or green house bioassay will prove to be ineffective at field conditions, it is recommended that doses used in laboratory or green house should be doubled for field application.

For soil application, field foliage-doses should be doubled. In case of organic mug soil, double dossage should be used, as corresponding to normal soil; this turns out to be true for all organic insecticides.

For high and low volume spray pumps, mesh advisable for filters goes between 50 and 100 mesh.

In case of wettable powder, mechanical agitation system is necessary.

In order to have right pressure lectures in machines containing big installations for spraying (booms), should have pressure guauge measured near the end of the tube. For handling booms, check carefully distance of nozzle to vegetals and distance between nozzle. Maintain, for example 80^{o} cone evenly in all nozzles. Use always same angles, through out all experiment. High pressure will equalize application, whereas low pressure will render defective application.

Technology of application is selected according to formulation in case-by-case basis : for example, for herbicide application, flat spray nozzle, which rnders hollow cone, is satisfactory. For disease and pest control, even spray nozzle, which render solid cone is more effective.

For more detailed information various aspects of technology of application of pesticides we refer to the book of Mr. G.A. Mathews (1979), London, Longman, 334 P.

COLLECTING DATA IN FIELD CONDITIONS

Data register, handling and analysis

1) Field bound book, the better. Keep always in safe place. Put in everything.

2) For some cases, handout sheets prepared specifically for each case may prove very practical and useful but should be careful not to loose them. In case of field note sheets, advisable to recopy all data in a bounded note book.

3) "Field book" should be made in such a way that any body may understand what is going on in the field.

4) Note books made of squared paper are more practical since helps for making graphs and maps.

PRESENTING FIGURES AND GRAPHS IN SCIENTIFIC REPORTS

When we are supposed to reduce the size of a table or graph, we should be aware of the proportions, because apparently reading messages are more affected than graphs: i.e. if we reduce the graph to half original size, the letters will be reduced about 4 times.

When graphs are drawed, it is suggested to use the range in ordinate exactly right to contain the curve, avoiding extra space.

Also the scale in the ordinate should be carefully selected, because same graph may look different only by c changing the scale.

Comparison of indppendent groups of things may be presented in graphs of bars. Avoid full black stripes, prefer datched bars.

Histograms are suggested only when variance is great.

ANALYSIS AND REPORT OF CONCLUSIONS

Analysis of Variation is applied for experimental data, and discriminative test (DMS, Duncan, Turckey) will show the differences among treatments. Computer canned programmes are available everywhere for data processing and analysis.

Also, LD 50 PoloLeora soft-ware programme is available at PDC for data processing and analysis of dose mortality and time mortality relationships.

UNIDO COMMENTS

The report gives in great details measures that are to be taken by the project authorities to establish a reliable insect breeding area at PDC. The big problem could be the extreme temperatures in Delhi, humidity variations and the power failure and to overcome these drawbacks a good insect breeding station in Delhi would be very expensive and hence they should make use of the facilities available in other institutions and have only the basic insect rearing facilities.

The author has given a list of books to purchase and also a list of institutions to contact for training. The list of equipment given should be carefully selected based on the needs.

Experience in the author's home country could be useful to Indian conditions.