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UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION
Vienna International Centre, P.O. Box 300, 1400 Vienna, Austria
Tel: (+43-1) 26026-0 · www.unido.org · unido@unido.org

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PESTICIDE DEVELOPMENT PROGRAMME IN INDIA

DP/IND/80/037

INDIA

Technical report: Biocide Development*

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 M.S. Mulla,
consultant in biocide development

Backstopping officer: B. Sugavanam, Chemical Industries Branch

United Nations Industrial Development Organization
Vienna

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* This document has not been edited.

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Objectives and Contacts:

The objectives of this consultantship were to:

- Hold discussions on work and studies carried out at PDPI on pesticide formulations especially with regard to the development of the biocide Bacillus sphaericus 1593 M and other strains.
- Present lectures on the recent developments of biocides, their formulations and their prospects in insect control programs in developed and developing countries.
- Assist in the development of bioassay techniques of new formulations of biocides developed in the Centre.
- Provide advice on the possible commercialization of promising formulations of biocides.
- Suggest equipment and training needed to carry out further work in the area of biocides.
- Submit a report including recommendations on future developments and programmes at PDPI.

This consultant visited PDPI Centre (Udyog Vihar, Gurgaon, Haryana, India) from October 28, 1988 to November 20, 1988 inclusive. Technical and informal discussions were held with the manager and scientific staff

at the Centre. Contacts were made and discussions held with the following scientists:

Dr. S. K. Khetan, General Manager (R & D) PDPI

Dr. S. P. Bhateshwar, Entomologist, Testing and Evaluation

Dr. Y. P. Ramdev, Jr., Entomologist, Testing and Evaluation

Mr. S. P. Yadav, Superintendent Testing and Evaluation

Dr. R. K. Khandal, Group Leader, Clay Minerology, Physician Chemical Properties Group Leader

Dr. P. K. Ramdas - Group Leader Formulation Technology, Physical Analysis

Dr. S. Y. Pandey - Group Leader Analytical Chemistry and Instrumental Analysis

In addition, several staff of UNDP-UNIDO were contacted and discussions on further development and training of personnel at PDPI were carried out. These were:

Mr. M. Islam, Unido Senior Industrial Development Field Advisor (SIDFA)

Mr. M. Ramachandran, Senior Program Officer, UNDP

Mr. Sat Pal, Assistant Program Officer, UNDP

Others with whom I met and gained background information were:

Dr. H. K. Khan, Secretary, Department of Chemicals and Petrochemicals, Ministry of Industry

Dr. S. P. Dhua, Chairman and Managing Director, Hindustan Insecticides, Limited.

Mr. D. R. Sharma, Deputy General Manager, HIL

MALARIA RESEARCH CENTER (MRC)

INDIAN COUNCIL OF MEDICAL RESEARCH

A detailed visit was made to MRC to gauge and assess the nature and scope of research by that agency on the development of biocides for the control of mosquito vectors of malaria and filariasis. This research unit has proposed and is planning to undertake large-scale field trials on two biocides (Bacillus thuringiensis ser. H-14 and B. sphaericus) for the control of Anopheles and Culex mosquitoes. A visit to the test villages in the vicinity of Delhi was made along with the technical staff of MRC. Mosquito breeding sources were assessed and procedures and protocols for the conduct of the 3-year program were discussed with the scientific and technical staff of this unit.

This large-scale field trial program on biocides will be the first one of its kind anywhere in a developing country. The proper implementation of the research program will generate a great deal of needed data on the potential uses of biocides in operational programmes aimed at suppressing disease vectors. Over the long haul, the expertise and process technology of PDPI will play a major role in the production and formulation of biocides needed in operational vector control programs in India. A strong link currently exists between PDPI and MRC and further collaboration between the two will be mutually beneficial.

At the invitation of Dr. V. P. Sharma, Director of Malaria Research Center, I presented a seminar on "Ecological Basis for the use of Biocides in Vector Control Programmes." Following this seminar discussions were held with the technical staff and postgraduate students. I found the caliber of the scientists and the nature and scope of scientific research programs to be of a very high quality. MRC

in my opinion is a great source of scientific knowledge with regard to the epidemiology of malaria and control of mosquitoes and this unit will and should play an important role in the development of biocides in vector control programmes.

DEVELOPMENT AND MANUFACTURE OF GENETICALLY ENGINEERED MICROBIAL PESTICIDES

This is a newly conceived project which may be submitted for funding to UNDP by PDPI. The main objectives of this project are to: use new biotechnology for increasing the productivity of agricultural commodities and to facilitate control of insects of public health importance. The immediate objectives are to develop potent strains of the microbial control agents Bacillus thuringiensis for crop protection by genetic manipulations, and to improve the potency of another microbial control agent B. sphaericus for use as a larvicide against mosquito vectors. The project also lists some other objectives which could be realizable recognizing the expertise, equipment and facilities at the disposal of the Centre. These objectives are:

1. Development and standardization of formulation parameters for commercial exploitation of biocides.
2. Safety tests on biocides.
3. Field evaluation of biocides with an assessment of impacts on nontarget biota.
4. Scale-up studies leading to commercial production of biocides.

It should be pointed out that the development and commercialization of microbial control agents will provide some safe and economically feasible alternatives to the use of hard-core and relatively toxic pesticides for the control of phytophagous insects as well as insects of public health importance. Therefore development and exploitation of these biocides should be accorded highest priority in developing countries.

As far as this project (genetic engineering) is concerned, it needs some modification and redirection. The bioengineering and genetic cloning of toxin genes could best be done by others. PDPI with a primary mission of development and application of pesticides and their formulations has personnel equipment and facilities to concentrate on the more applied aspects of the project. The project was discussed in details with Dr. Khetan and we both agreed that PDPI will be in much better position to concentrate on the development of practical and economically feasible production processes for biocides and to plan in-depth studies on the development of specific formulations of the raw products or technical materials and to implement studies to test and evaluate the biological activity and efficacy in laboratory, simulated field experiments and small-scale field trials. For large-scale field trials under a variety of conditions in the subcontinent, PDPI should seek and establish linkage with other institutions in agriculture and the health sector.

TRAINING PROGRAM

QUALITY CONTROL OF PESTICIDE FORMULATIONS

This Training Programme sponsored by UNDP/World Bank/FAO/UNIDO was held at PDPI during 1 November to 4 December 1988. The programme and activities of this Training Program are presented in Annex I. Participants from several RENPAP (Regional Network on Pesticides for Asia and the Pacific) countries attended this program. The scientists and staff of PDPI, consultants from the World Bank, UNDP and UNIDO and many scientists from organizations and institutions within India delivered lectures and held demonstrations and practical exercises (see Annex I).

Although I was not aware of this Training Programme until my arrival in Delhi, I was fortunate to be asked by the organizers to participate in this worthwhile programme. This participation indeed was one of the highlights of my visit. I chaired two sessions of this training programme and delivered two lectures (see Annex I):

1. Pesticide quality assurance as related to biological activity (Annex II).
2. Bioassay of insect pathogen formulations (Annex III).

I found this training programme to be one of the best held in a developing country, imparting a great deal of relevant basic and applied information on pesticides and their formulations. PDPI is one of a very few organizations in the world that can plan and successfully hold such a training program. For the RENPAP as well as other developing countries, training programs of this type are a must for the development and judicious and proper use of pesticides and pesticidal formulations.

BIOLOGICAL ASSESSMENT GROUP AT PDPI

At the present time this unit consists of three professional scientists (two with Ph.D. degrees and one holding MSC degree) and 3 assistants. All the professional scientists have had or will have training and educational opportunities in UK and US universities. This level of personnel resources seems to be adequate for the time being to take care of needed biological work.

The professional and subprofessional staff in this unit are quite capable to design, develop and implement tests on biological evaluation of technical and formulated materials both in the laboratory and under simulated field conditions. There are, however, some stringent limitations hampering the productivity and progress of this group. This will indeed be true if PDPI future plans of expanded activities in the area of the development of biocides and other groups of pest control agents are materialized. These constraints in terms of the need for suitable facilities will be discussed later on.

At the present time PDPI has limited number of target insects under colonization. This number has to be increased in the future for developing biological tests on broad range of target insects and disease vectors. The unit currently has colonies of the following injurious insects and disease vectors:

Lepidoptera: 3 species, leaf feeding caterpillars

Diptera: 3 species, housefly, Drosophila and a mosquito

Coleoptera: 1 species. Tribolium species

Attempts are made to establish a colony of phytophagous mites and a species of aphids.

In discussion with the staff, it was recommended that the unit attempt to establish the following species of insects and disease vectors in addition to those currently in culture.

1. Three species of mosquitoes, preferably Aedes aegypti, Anopheles stephensi, and Culex quinquefasciatus, both with widespread distribution in tropical countries.
2. Establish a colony of the cockroach Blatella germanica, as this is one of the most important pests in domestic situations, restaurants, hotels, food processing plants, etc. A great deal of effort is expended to control this pest in urban areas. The colonies could be a source of infestation into the buildings, so care must be taken to maintain these in a building away from the main cluster of buildings.
3. Establish one or two additional species of stored product pests so that both external and internal breeders and feeders are represented.
4. It would be desirable to establish a colony of a nontarget species to conduct safety tests on a standard organism cultured in the laboratory. A species of the genus Daphnia should suffice for this purpose.

Facilities: Although the size of rooms and the total usable space allocated to this group are quite adequate for biological research and development, the space, however, is not totally suitable for colonization of insects and testing of bioactive compounds and their formulations. In discussions with the technical staff the following modifications and alterations in space are deemed essential:

1. Construct at least 4 culture rooms in the existing set up. These rooms should be provided with drop ceilings (existing rooms have very high ceilings and are not amendable to temperature and humidity control), temperature control and a regimen of photoperiod required for raising host plants or insect cultures. For mosquitoes there is a need for light gradation to provide dusk and dawn effects. These conditions are necessary for colonization and culture of many insects.
2. Two rooms are needed for testing and evaluation which should be used solely for testing and bioassay of bioactive materials and formulations. These rooms should be provided with hood and exhaust fans.
3. Glasshouse construction is a must for growing a variety of host plants and their pests. PDPI is ideally located with ample space to develop glasshouse facilities on its campus.
4. Construction of small mesocosms (ponds) where efficacy, persistence and fate of pesticides and their formulations could be studied.

BIOASSAY AND TESTING OF TECHNICAL MATERIALS AND FORMULATIONS

The biological assessment unit is capable to carry out studies on the bioassay of a variety of products including biocides. With the availability of suitable testing and holding rooms the unit will be able to carry out a variety of tests, providing valuable information and feed-back to the formulation Research and Development group, who are involved in product development, formulations, quality assurance etc

Bioassay techniques, protocols and procedures were discussed with the staff. A general procedure for bioassays of pathogens is included in the Annex III. It is important that bioassay studies should contain complete information about the formulations, manufacturer, code number and lot numbers. Additional information on species, strain, instar and origin of test species and hosts should be included.

All concentrations and preparations should be tested in replications. Each treatment and control should be replicated at least twice in each test and the tests on each product should be run on two or three different occasions. This will provide for the biological variability of test species on different days and yield a minimum of 4 replicates for a given treatment and control.

FORMULATIONS OF PESTICIDES

Pesticides are produced as technical materials having high purity. These products are produced as gaseous, liquid (viscous or nonviscous), semisolid and solid (amorphous, crystalline or waxy). Most of the synthetic pesticides are soluble in organic solvents with little or no solubility in water. Technical materials of these products can be tested as dilute solutions, emulsions or suspension with or without water as a carrier.

Formulations of synthetic pesticides commonly available are:

- Solution or soluble concentrates composed of the technical material dissolved in organic solvents.
- Emulsifiable concentrates containing technical material dissolved in an organic solvent to which a surfactant or other adjuvants are

added. These concentrates are readily diluted with water and applied as aqueous sprays.

- Flowable concentrates composed of technical material suspended as particles in organic or water phase. These concentrates are also diluted with water prior to testing and application.
- Wettable powders containing the active ingredient, inert dust, such as clays and others and a wetting agent. The powders are suspended in water prior to testing and application.
- Dusts are dilute formulations containing technical materials and inert diluents. Dusts are used directly without further dilution.
- Granules are like dusts but consisting of much larger particles than the dust. Granules are applied directly without further mixing or dilution.

Additional formulation of pesticides are aerosols, slow-release and baits. Each one of these and above formulations are prepared for specific requirements.

Test and bioassay methods have to be appropriately developed for each type of formulation. In general, the technical material and its emulsifiable, flowable concentrates and wettable powders are the most common products tested during the initial development phases. Subsequently, other specialty and tailor-made formulations will be screened and bioassayed. It is imperative that the activity of the technical materials and the most commonly used formulations be determined before tailor-made specific formulations are studied for bioactivity.

DEVELOPMENT AND COMMERCIALIZATION OF BIOCIDES

During the past 3 decades or so, synthetic pesticides proved to be highly effective against a variety of pests belonging to various taxa. However, due to the appearance of acquired resistance and environmental concerns, many of the highly effective pesticides are no longer used in many pest and disease vector control programs. Recently, greater emphasis has been placed on developing alternative strategies employing cultural control methods, environmental management and the use of biological control agents especially microbial control agents.

Among the biocontrol agents, viruses, spore-forming bacteria and fungi offer some promising alternatives. These microbial control agents serve as supplementary to other control methods and could be effectively used in an integrated pest management program against a variety of crop pests and disease vectors.

Among the microbial control agents, the spore-forming bacteria have attracted a great deal of attention especially for the control disease vectors such as mosquitoes and black flies. The spore-forming bacteria Bacillus thuringiensis ser H-14, was recently shown to have activity against numerous species of mosquitoes and black flies. This pathogen is currently employed for the control of mosquitoes and black flies in many countries of the world. Another subspecies of this bacterium known as Bacillus thuringiensis subspecies kurstaki has been used for the control of agricultural and forestry pests for the past two decades or so.

Another spore-forming bacterium Bacillus sphaericus has been recently found to show high level of activity against many species of mosquitoes, especially those belonging to the genus Culex.

Characteristics of Microbial Pathogens - Bacteria:

The spore-forming bacteria Bacillus thuringiensis and B. sphaericus produce toxins in the spore and parasporal bodies. The spores are resistant to desiccation and temperature and they can survive for prolonged periods in soil. These pathogens can be produced locally and commercially in many countries where food and fermentation process technology is available. One important advantage of these spore-forming bacteria is the short turnover time, where maximum spore and toxin production is achieved in 27-40 hrs.

These pathogens can be produced in small fermenters (50 to 100 litre capacity) as well as commercial scale fermenters with 20 to 50 thousand litre capacity. Another important feature of these microbial agents is that they can be readily formulated and used alone or in combination with other control strategies against a variety of pests and disease vectors.

Formulations of Microbial Control Agents

Microbial control agents produced by fermentation technology consist of vegetative cells, spores with toxin particles and nutrient materials added for the growth and production of the pathogens. At the present time these pathogens are produced as semi-solid or liquid materials. Most fermentation processes yield liquid slurries of these pathogens. The slurries can be formulated as liquid or solid formulations.

The technical products of microbial control agents are composed of complex materials and are insoluble in organic solvents or water. Therefore, the slurries have to be processed and formulated in such a

way that the formulations are easily suspended in water used as a carrier in the application of the products. To obtain suitable formulations of microbial control agents, new technology and formulating processes have to be developed for producing appropriate and effective formulations.

The most commonly available formulations of microbial control agents at the present time are:

1. WP or WDP - Water dispersible formulations.
2. Liquid as FC - flowable concentrate formulations
3. Granules generally prepared on clay base.
4. Granules prepared on corn cob and other agricultural by product materials.
5. Dissoluable granules which are suspendable in water. The use for this formulation has not found widespread acceptance.
6. Controlled release formulations which provide for somewhat long-lasting control. These formulations are prepared as briquettes, donuts, and encapsulated materials.
7. Mixture of pathogens and other pest control agents such as monolayer filrus and insect growth regulators.

Role of PDPI in the Development and Commercializations of Biocides

Current efforts in research and development of biocides for the control of pests and disease vectors in India is quite inadequate in contrast to the effort made in other developing countries. Since biocides could provide a practical alternatives to the hard-core pesticides in an integrated pest and vector control technology, it is essential that systematic efforts be made to develop schemes for the

development, production and marketing of promising biocides. PDPI with its excellent resources of trained personnel, state-of-the-art equipment and relatively good facilities should take the initiative to spearhead this programme. It should be noted that the effort of many research and development organizations will be needed to advance the production and utilization of natural biocides. Many university researchers, scientists at research institutes and centers (in agriculture and public health), pesticide manufacturing and formulating concerns and fermentation technologies have to be involved.

At the outset, PDPI could play an important role in small-scale production of biocides. PDPI is in an excellent position to research and develop some of the commonly used formulations as well as tailor-made formulations which may be needed for the control of specific pests and disease vectors in specific situations. Currently available biocides and natural products as well as those to be developed in the future require systematic studies and development efforts before their use can be expanded in pest and disease vector control programmes. PDPI will and should play a major role in the development and exploitation of biocides.

GENERAL RECOMMENDATIONS

Development and Commercial Production of Entomopathogens

Pesticides Development Programme India (PDPI) has the capability in terms of personnel resources, equipment and plant facilities to initiate research on the development and production of microbial control agents. PDPI needs to diversify and broaden the scope of its operation to cover a variety of disciplines dealing with the development,

production and formulation of synthetic pesticides as well as natural biocides.

It is therefore recommended that PDPI:

1. Develop a programme of research and development on the production of entomopathogenic bacteria, which have potential for the control of crop pests and disease vectors.
2. PDPI should recruit a microbiologist and the necessary support staff to provide a base of operation for assuring the quality control of entomopathogens and their formulations.
3. PDPI should develop suitable laboratories and acquire the necessary equipment to facilitate local production of entomopathogens where production parameters such as nutrients, time profile, potency and product suitability can be studied.
4. PDPI should in a matter of 2 to 3 years develop and upscale production strategies for producing large quantities of entomopathogens.
5. Systematic and concerted effort should be made to prepare appropriate formulations of entomopathogens using locally available materials.
6. PDPI should expedite laboratory testing and evaluation of technical as well as formulated materials against numbers of representative taxa of crop pests and disease vectors.
7. PDPI should plan for and undertake small-scale field tests to determine the efficacy of technical and formulated materials against important crop pests and disease vectors.
8. Large-scale field trials with formulated materials should be carried out by other research centers or organizations. PDPI

should provide the impetus for such trials and develop collaborative arrangements with other appropriate units.

9. Greenhouse, culture rooms and testing facilities should be improved and developed for biological testing and evaluation. At the present time these facilities do not exist, or are not appropriate for conducting a variety of tests.

10. Training programmes should constitute one of the ongoing activities of PDPI. This centre could serve as a training base for students and experts of the RENPAP countries where appropriate training and education in pesticides, their formulations, quality-assurance, standardization and analytical procedures can be carried out.

11. In addition, the scientific and technical staff of PDPI will need to update their training and education in the area of their expertise from time to time. It is highly recommended that PDPI staff be given the opportunities to visit other research and development organizations abroad for a period of 3 to 6 months.

ACKNOWLEDGEMENTS

My sincere thanks and appreciation to UNIDO offering me the opportunity to take this assignment. Sincere thanks also go to the numerous staff and administrators of UNDP (Delhi) who ably assisted me in discharging my assigned responsibilities. Dr. S. P. Dhua, Chairman and Managing Director, Hindustan Insecticides, Ltd. was instrumental in providing background information on the activities and scope of oepration of HIL. Dr. S. K. Khetan, General Manager (R and D), Pesticide Development Programme India was extremely helpful in availing

the necessary backgound information and in discussing future plans of research and development. Without this in-depth interaction with Drs. Dhua and Khetan, it would not have been possible to discharge the assigned tasks.

UNDP/WORLD BANK/
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Regional Network on Pesticides for Asia and the Pacific

Training Programme on
Quality Control of Pesticide Formulation
1 November-4 December, 1988

Pesticide Development Programme India
Udyog Vihar, Gurgaon, India-122 016.

PESTICIDE QUALITY ASSURANCE AS RELATED TO BIOLOGICAL ACTIVITY

by

Mir S. Mulla

Department of Entomology
University of California
Riverside, California 92521, USA

Most pesticides are produced as technical materials where the active ingredients constitute 90% or more of the product. Since only very small quantities of the active ingredients are needed for the control of specific pests, it is not physically possible to distribute or broadcast such small quantities of pesticides evenly over the infested areas. Therefore it is necessary to formulate the technical materials into a system that can be diluted with a carrier and applied in pest control in large volumes as sprays or in sufficient quantities as dusts or granules to facilitate good coverage of the area where the target pests prevail.

The synthetic pesticides are produced as technical materials and in solid, liquid or gaseous state. The solid material could be either amorphous, waxy or crystalline. The liquid products could range from highly viscous thick liquids to non-viscous materials. All these products including the gaseous pesticides are seldom applied as technical materials. They have to be properly formulated and applied with appropriate equipment to the targetted area. Availability of suitable formulations facilitates easy application of pest control agents to large areas of infested commodities and premises with currently available equipment. Through the use of appropriate formulations it is also easy to obtain complete coverage of target areas.

3. Biological Pesticides - Natural Products

At the present time these pesticides occur in only a few of the lower clases of organisms. Viruses and spore-forming bacteria are the two main groups of biological pesticides. Some fungi are also now being developed for pest control. Although a number of parasitic nematodes have been studied for insect control, no commercial exploitation of these has been achieved as yet.

Among the biological control agents of insect pests and disease vectors, two spore forming bacteria have been employed on a commercial, semi-commercial scale. Several strains of the common bacterium Bacillus thuringiensis (Bt) have been developed for pest control. Bt strain Kurstaki has been used for many years to control lepidopteran pests of forests, vegetables and fruits. Another strain Bt (H-14) was recently developed for the control of pest and vector mosquitoes and black flies breeding in rivers and streams.

Another highly effective spore forming bacterium Bacillus sphaericus has been widely studied against mosquitoes. This spore-forming agent produces crystalline parasporal bodies which on ingestion by mosquito larvae cause mortality at extremely low concentrations.

The spore-forming bacteria are ammenable to local and commercial production. They are quite safe, have little or no environmental implications. Their use should be recommended whenever and wherever they show biological activity. Production, commercialization and formulation of these microbial control agents should be promoted wherever possible especially in developing countries.

soluble concentrate formulations are employed as low or ultra-low volume applications for the control of pests in crops, forestry and insects of medical and public health importance. They can be applied with both ground equipment and aircraft.

2. **Emulsifiable Concentrates** : These are commonly used formulations in insect control programme. The formulations are prepared from the technical material which constitute anywhere from .10 to 70% in a suitable organic solvent generally derived from petroleum distillates. To make these formulations miscible with water the cheapest diluent and carrier; these formulations contain 2 to 10% of a surfactant or a number of surfactants depending on the requirements of a formulation for the control of pests in a variety of habitates.

3. **Flowable Concentrates:** These formulations are prepared from solid or liquid materials where the particles of the technical material or a formulation are stabilised and suspended in aqueous phase or oil phase. In addition to the technical material and solvents, adjuvants for stabilization and surface active agents for altering the surface tension are also incorporated

4. **Wettable Powders:** These formulations are the next commonest products after the emulsifiable concentrates. The formulation in general is composed of the technical material (solid or liquid) a diluent or carrier (clays, etc) and wetting agents. These formulatins are suspended or dispersed in water and applied for the control of crop pests and insects of medical and public health importance.

5. **Dusts** : These formulations consisting of the technical material (2-5%), diluent or carrier are applied directly to the target area without the use of water or other carriers. These formulations are usable in the control of crop pests as well as insects of public health importance.

bait contacted and consumed by the pests, induces mortality. The baits have the advantage that they can be used selectively in limited areas where the pest prevail. There is no need to get complete coverage with the baits.

Formulation Requirements:

There are a number of requirements as related to the application and biological efficacy of pesticide formulations. They should be stable in storage and homogenous. Other physical requisites are that the formulation should be flowable, not caking and viscous and they should be handled with ease.

Another important parameter is the miscibility of emulsifiable concentrate and flowable concentrate formulations and the suspensibility of WP formulations in water. These formulations should be amenable to be applied by ground equipment including but not limited to sprays, dusters, blowers etc. Solid formulations such as granules, briquettes and controlled release formulations should also have the above properties.

Most of the formulations prepared as EC, FC and WP formulations have uses in crop pest control for application to foliage, fruits and seeds and the root zones. Some of these formulations as well as other such as aerosols, smokecoils are used for the control of pests in domestic and household situation. These materials can be used as space sprays or aerosols, residual deposits and mixing with stored food. These and some other formulations are also employed for the control of insects of public health importance in indoor as well as outdoor situations. Insects of public health significance breeding in aquatic habits are readily controlled with some of these formulations.

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BIOASSAY OF INSECT PATHOGEN FORMULATIONS

by

Mir S. Mulla

Department of Entomology
University of California
Riverside, California 92521, USA

At the present time majority of the pathogenic organisms causing diseases and mortality in insects, belong to viruses, bacteria and fungi. Viral pathogens, currently known, attack lepidopteran and some other insect pests. In the bacteria group most of the pathogens currently known attack pests in the order Lepidoptera, Coleoptera and Diptera. Several groups of fungi are also known to cause diseased conditions in members of the order Coleoptera, Homoptera and Diptera. The entomopathogens affecting dipteran insects are used primarily for the control of mosquitoes, black flies and a few other insects. In this paper procedures and protocols for the evaluation of these pathogens showing activity against mosquitoes and black flies will be discussed. Emphasis will be placed on laboratory bioassays and procedures for 2 entomopathogenic bacteria (Bacillus thurigensis) ser H-14, and B. sphaericus) and the water mold Lagenidium giganteum and their formulations. Bt (H-14) a microbial larvicide as its agricultural counterpart, Bt kurstaki, is produced commercially and used in mosquito control programmes in various countries. It is also used for the control of Simulium black flies in West Africa and other countries around the world.

B sphaericus another spore - forming bacteria is highly efficaccous against several genera of mosquitoes. This entomopathogen is highly specific and studies on its efficiency and safety are adequately advanced to facilitate its registration in mosquito control programmes. The fungus L giganteum has also been extensively studied and its use in some mosquito control programmes may be forthcoming.

Pretreatment During the pretreatment period the following quality assurance elements are desired.

a) **Storage Stability:** Since formulations are not used right away, they should have reasonable sotrage stability especially under tropical conditions. Stability to product for a 2 years period for most pesticides is desirable.

b) **Homogenety:** The product should be homogenous, without separation or concentration of the active materials in parts of the package or container.

c) **Flowability:** Since formulations are mixed with diluents or carriers (e.g. water) before application, they should be flowing freely. Some dry formulations are applied directly without dilution and it is more important that these be highly flowable and easy to handle.

d) **Miscibility:** Since most EC, FC and WP formulations are applied as aqueous sprays, it is highly important that they mix with water thoroughly without much agitation.

e) **Application** : Formulation prepared for pest control should be easily applied with available equipment. Lack of proper equipment will result in spotty coverage, drift of the formulation and finally the availability of the toxicant to the target species.

Posttreatment Considerations

The post-treatment considerations influencing biological performance are as follows:

a) **Drift** : Drift is a major problem in the application of dusts and aqueous sprays. Some of the active ingredients can be

Granules - Prepared primarily on agricultural by products

Briquettes - Large chunks, used as controlled release materials

Encapsulated materials - either liquid or solid to improve stability and longevity of formulations.

3. **Why Bioassays :** Biocides such as viruses, bacteria, and fungi are living entities. Their spores, cells and toxin particles may enter the insect through cuticular penetration or by ingestion of the particles. Some of these pathogens multiply and proliferate through the host tissues, causing localized on systemic infection. In others such as the bacterial larvicides, the toxin particles ingested act on the midgut epithlium of susceptible species, causing disruption of the midgut wall. The toxin particles are composed of complex proteins having high molecular weights.

At the present time there is no practical chemical method to determine the contents of the active materials in the formulation. In the case of the infectius agents such as fungi, the formulation contains hyphae, spores or both. Therefore, none of these entomopathogens can be determined chemically. The potency of these agents has to be determined either by bioassays against suseptible targets or by microbiological methods where the spore or cell contents are assessed. Therefore, good practices in conducting bioassays are essential for assessing the potency and biological activity of biocidcs.

BIOLOGICAL ASSESSMENT - LABORATORY

Different bioassay techniques are employed for the evaluation of the activity of microbial control agents. The procedures employed will depend on the formulation of the pathogen and the species of target insect employed. Only a few species of the target insects are colonizable and therefore some bioassay may have to be done against wild-caught target species.

4.1 **Bioassay against mosquitoes :** The currently promosing pathogens are active against mosquito larve and not other stages. Therefore, technical and formulated materials are evaluated against larvae of various species that have been colonized in the laboratory or larvae obtained from field breeding sites. Third and early 4th instar larvae should be employed in tests, because the two bacterial agents which produce

4.5 Data collection and analysis : Mortality readings of all replicates for each concentration and dosage should be totalled and averaged (see attacvhed form). The mortality values thus obtained should be plotted against concentration and dosage response line established on probit log paper. The LC_{50} and 90 (lethal concentration for 50 and 90%) should be read off the dosage response line. These values may also be obtained from a computer utilising an appropriate soft-ware programme. The LC_{50} and LC_{90} values are expressed as mg/l of the active materials or formulations. In some cases the values may be given in terms of the spores or cells of the pathogen per ml of water. These values are relative, giving the potency of various materials or formulations which can be compared among different materials or formulations.

5. Preparation of standard test suspension : In order to test microbial control agents, it is necessary to prepare proper strength suspensions for the standard as well as the test material, suspensions are generally made in distilled on tap water. Suspensions thus prepared can be stored for upto a week in refrigerator. It is desirable to prepare a new stock suspension and its dilution every week.

The standard is generally prepared in a laboratory and its potency established against a species of mosquitoes. The potency is expressed in international toxic units per mg of product (ITU/mg) potency of other materials is expressed as spores or cells/ml.

The stock suspensions are prepared as 1% (wt/volume in g/100 ml) of the product in water. Only WDP, FC, dissolveable granules and technical materials can be made into suspensions. Granules, brickets and other tailor-made formulations not missible or dispersable in water have to be applied directly. Since these formulation can not be diluted, they have to be applied directly, thus necessitating the use of larger containers and volumes of water.

For testing suspensions, serial dilution have to be made (Table 1) with water so that aloquots of 0.1 to 2 ml of a given suspension yielding a desired range of concentrations, can be added to the test vessels. In each test bank and controls have to be run. The stock as well dilute suspentions can be stored for a period of a week or so in the refrigerator, but should be discarded and not used beyond a week.

TABLE 1

ml Aliquot of various strength solutions (% wt/Vol) added to 100 ml water to yield ppm (mg/liter)

Solution (%)	PPM	ml aliquot	final conc (ppm) in 100 ml
1	10,000	1.0	100.0
		0.5	50.0
		0.1	10.0
0.1	1,000	1.0	10.0
		0.5	5.0
		0.1	1.0
0.01	100	1.0	1.0
		0.5	0.5
		0.1	0.1
0.001	10	1.0	0.1
		0.5	0.05
		0.1	0.01
0.0001	1	1.0	0.01
		0.5	0.005
		0.1	0.001
0.00001		1.0	0.001
		0.5	0.0005

Volumes: 1 liter = 1000 ml, 1 ml = 1000 micro liter
1 cubic ft = 7.5 gal = 28 liters
1 gallon = 3785 ml = 4 qts = 8 pints = 128 oz

Surfaces: 1 hectare = 10,000 m^2 = 2.2 acres
1 acre = 43560 ft^2
1 sq ft = 0.111 sq yard = 0.83 m^2

Lengths: 1 Km = 0.62 mile = 1,093 yds
1 m = 39.7 inches
1 inch = 2.54 cm = 0.0254 m
1 ft = 0.333 yd = 0.3048
1 yd = 91.44 cm = 0.9144 m
1 M (statute) = 1,760 yd = 5280 ft = 1609.3 m

Weights: 1 pound = 0.454 Kg
1 kg = 2.2 lbs
1 g = 0.035 oz

Conversions: in^2 to cm^2, multip'y by 6.5
yd^2 to m^2, multiply by 0.8
ft^2 to m^2, multiply by 0.09
acres to ha, multiply by 0.4
$miles^2$ to Km^2, multiply 2.6