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INDIA

Technical report: Development of Bio-pesticides
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 Mr. Marshall Laird, Biologist

Backstopping officer: B. Sugavanam, Chemical Industries Branch

United Nations Industrial Development Organization
Vienna

* This document has not been edited.

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Abbreviations

UNDP	United Nations Development Programme
UNIDO	United Nations Industrial Development Organization
PDPI	Pesticides Development Programme India
RDC	Research Development Centre
ICMR	Indian Council of Medical Research
MRC	Malaria Research Centre
NTO	Non-target Organisms
HIL	Hindustan Insecticides Ltd
WHO	World Health Organization

Itinerary of the Mission

15.08.1988	Departure from New Zealand
17.08.1988	Arrival in New Delhi and visit to UNDP office
18.08.1988	Discussion with Dr. Dhua, Chairman & Managing Director, Hindustan Insecticides Ltd and visit to PDPI Centre at Gurgaon
19.08.1988 to 16.09.1988	Worked at PDPI Centre
(ecept for 3 days)	
	In addition, visited
23.08.1988	Malaria Research Centre, Indian Council of Medical Research
24.08.1988 } 25.08.1988 }	Visit to field stations

I. WORK CARRIED OUT AND FINDINGS

1. Introduction

Initially my interest was to gain personal familiarity with the larval mosquito habitats in order to better comprehend the range of field circumstances under which "microbial insecticide" formulations developed at the Research and Development Centre (RDC/HIL) might best be targeted to specific practical control situations, with attention to field efficacy and safety concerns - especially those pertinent to nontarget organisms (NTOs). Such experience would also give me opportunities for defining a selection of such actual "breeding places" for relevant long-term field experimentation by the entomologists of RDC/HIL whose activities at present are centred on laboratory studies; defining them, that is, within the new, globally applicable 11-category mosquito larval habitat classification proposed in my new book, "THE NATURAL HISTORY OF LARVAL MOSQUITO HABITATS" (Academic Press, London and New York, 1988).

2. Field Trips

Accordingly, two field trips were undertaken with two entomological research officers of MRC/ICMR, Mr C.P.Batra and Mr C.R.K.Pillai. It was unfortunately not possible for the RDC/HIL entomologists to accompany us on the first of these. Undertaken from 06.30 until the early afternoon of 24 August, this gave me a unique opportunity of seeing the urban and semi-urban *Culex quinquefasciatus* and *Anopheles stephensi* production potential posed by the very heavy late-monsoon rains of 20 August, in South and East Delhi. The second field trip, in which, besides the two MRC/ICMR research officers just mentioned Drs Bhateshwar and Ramdev of RDC/HIL participated, primarily concerned larval habitats of *Anopheles culicifacies* in rural and semi-rural areas. It took us to the Ghaziabad District of U.P. (River Hindon), and Sadopur Village (PHC Dadri, U.P.) 25km east of Delhi. Returning to the latter we visited *A.stephensi* deep-well production sites at the suburban village of Madanpur Khadar and in the DIF Qutab Enclave, Phase 2; and a marshy larval habitat of *A.subpictus* (one of India's most abundant anophelines which is not, however, a major biter of humans nor of importance as a malaria vector) on the Agra Canal. Surprisingly, too, the vertical-sided, vegetation-free tanks of the new Baha'i Temple, proved to harbour many second instar larvae of *A.culicifacies* at the edges and in fact beneath the water jets which notably agitate the surface; this, despite the presence of a considerable population of predacious Hemiptera (Notonectidae, "water boatmen"), which are under investigation at MRC/ICMR as potential biocontrol agents for use against larval mosquitoes.

The dilemma in the latter connection is that while normally winged Notonectidae will undoubtedly devour mosquito larvae when these are fed to them in the absence of a diversity of alternative prey under laboratory conditions, they may well prefer other freshwater organisms to Culicidae in field situations. Moreover, it is wellknown that "water boatmen" readily disperse from one aquatic habitat to another. This circumstance undoubtedly detracts from their potential as practical biocontrol agents for deliberate introduction (but flight-polymorphic species* might work?). Nevertheless Notonectidae are certainly among the complex of predators in certain categories of water bodies, that collectively contribute to the natural regulation of numbers in mosquito populations. They thus serve as a good example of why efforts must be redoubled to develop practical integrated procedures for larval mosquito control, that will not adversely affect natural population-regulatory factors as do non-selective synthetic organic chemical larvicides. Thus while the latter remain essential for the residual treatment of inner walls of occupied buildings against adult mosquito pests and disease vectors, the far more specific "microbial insecticides" such as Bacillus thuringiensis ssp. israelensis and B.sphaericus (see following section) are much to be preferred as larvicides as they do not endanger Notonectidae and other such already-occurring generalist predators.

3. Work at PDPI, Gurgaon

As already indicated, the greater part of my time was spent each day at HIL's Research and Development area, Gurgaon. There I liaised with the professional staff whose skills were closest to the post's subject. There were many opportunities for exchanges of views with Drs Bhateshwar and Ramdev, both of whom had time to study the advance copy of "The Natural History of Larval Mosquito Habitats", which fortunately reached me from London (where it was published early in August) just days before my departure from New Zealand . Once they were familiar with its contents concerning the proposed standard "breeding place" classification therein. we had a slide projection session so that I could screen for them examples of each of the eleven broad categories of larval mosquito habitat from various parts of the world, as additional background to their reading and the MRC/ICMR field trip in which we had participated on 2 September. I also screened for them slides illustrating the development and large-scale operational trial of an integrated mosquito control methodology (combining source reduction with full community participation, with the joint use of the microbial insecticide, Bacillus thuringiensis ssp.israelensis in the Sandoz TEKNAR(TM) liquid formulation, and the insect growth regulator methoprene in the form of ALTOSID® (Zoecon) slow-release

*Such as the flightless phase of New Zealand's Anisops assimilis briquettes; together with the spraying of a residual adulticide to al inner walls. This project was supported by Canada's International Development Research Centre, and the South Pacific Commission during 1981-83. It was under my personal direction and was implemented by a joint field team from my former Research Unit on Vector Pathology (Memorial University of Newfoundland, Canada), and tropical Polynesia (our medical epidemiologist was a Tokelau Islander, and the Health Inspector for Tuvalu participated throughout). Three of the isolated atolls of the Republic of Tuvalu (05-11°S, 176-180°W)comprised the field site, with the capital and administrative centre of the group (formerly known as the Ellice Islands), the island of Funafuti (population c 2,000), chosen for the operational trial in 1983. The integrated methodology was designed to so lower the population of the domestic water-utilizing vector of dengue haemorrhagic fever, Aedes aegypti, as to break the chain of transmission of this disease. The venture's successful outcome has been fully reported elsewhere (Laird, M., Mokry, J., Semese, A. and Uili, R., 1985, Integrated control operations against Aedes aegypti in Tuvalu, Polynesia, pp.395-428 in "Integrated Mosquito Control Methodologies", Laird, M. and Miles, J.W., Editors, Vol.2, Academic Press, London and Orlando). Being the largest fully integrated such project undertaken up to that time, and representing the first-ever application of a microbial insecticide and an insect growth regulator to the drinking-water supply of an entire human community, it served as an appropriate example of the integrated approach to mosquito control destined to help solve Indian vector control problems concerning malaria and filariasis, for example, once microbial insecticide formulations developed for application to larval habitats of the appropriate categories, are in mass production in adequate quantities. A second reason for dwelling on this example during my assignment was the practical evidence it provides of the uniquely high levels of health and environmental safety associated with formulations (which as indicated can even be used as larvicides in potable water) based upon entomopathogenic bacteria.

Questions of Safety of Microbial Insecticides (the title of a forthcoming multi-author, internationally representative book which I have just completed editing - with the assistance of Drs L.A. Lacey and Elizabeth W. Davidson of the USA - for CRC PRESS,INC.,Boca Raton, Florida) were fully discussed with particular reference to Bacillus sphaericus and B.thuringiensis ssp.israelensis, between the General Manager (R.& T.) of RDC/HIL, Dr S.K.Khetan, and me. I was able to furnish him with relevant up-to-date information and references to the literature; and both through him and directly, liaison was maintained with HIL's Managing Director, Dr S.P.Dhua. All evidence points to B.sphaericus being fully as acceptable from both health and environmental standpoints as B.thuringiensis sspp. and the remainder of my time was spent in consideration of ways in which formulations of both might be maximized for efficacy against Indian vectors of malaria and filariasis.

Dr P.K.Ramdas and his group provided laboratory demonstrations of the B.Sphaericus formulations developed here. I found the self-spreading oil B.S.(1593M) 20SSO, particularly impressive. Action macrophotography conducted with the laboratory's instrumentation and fast (ASA 400) film that I had brought with me, furnished the sequence of illustrations presented hereunder (in practice the effective doasage is calculated as 5µl/ft²).-

Dropped onto the water surface, formulation 20SSO instantly spreads into a micro-reticulum, seen in fig.1 at a magnification of 7.5x

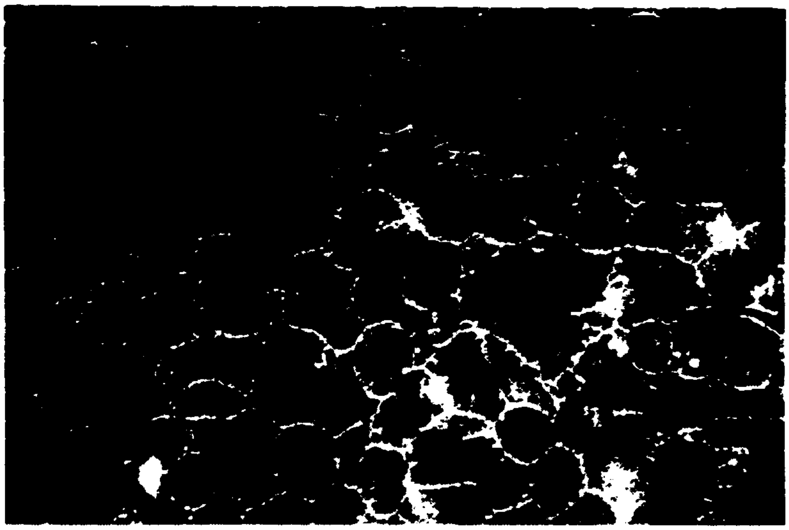

Fig.2, micro-reticulum 15x

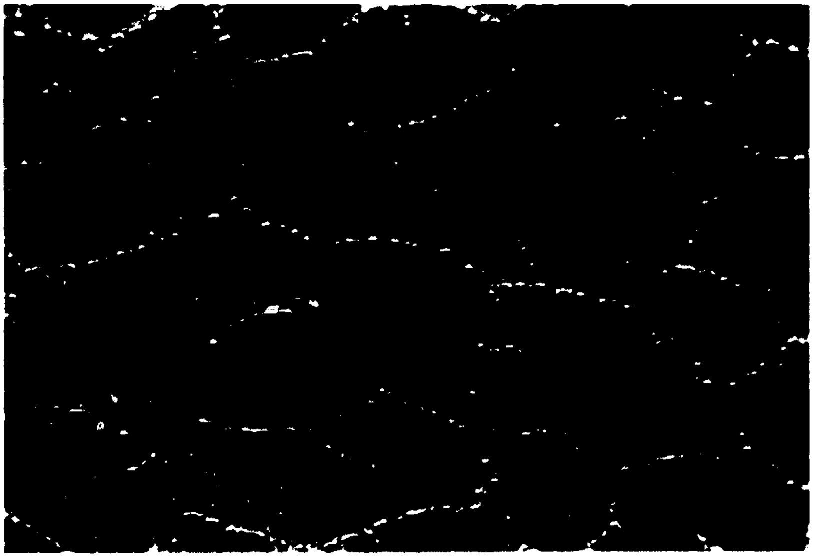

Fig.3, micro-reticulum
50x

Very soon after the micro-reticulum spreads over the surface, mutual repellency of its threads causes it to fragment into discrete globules.

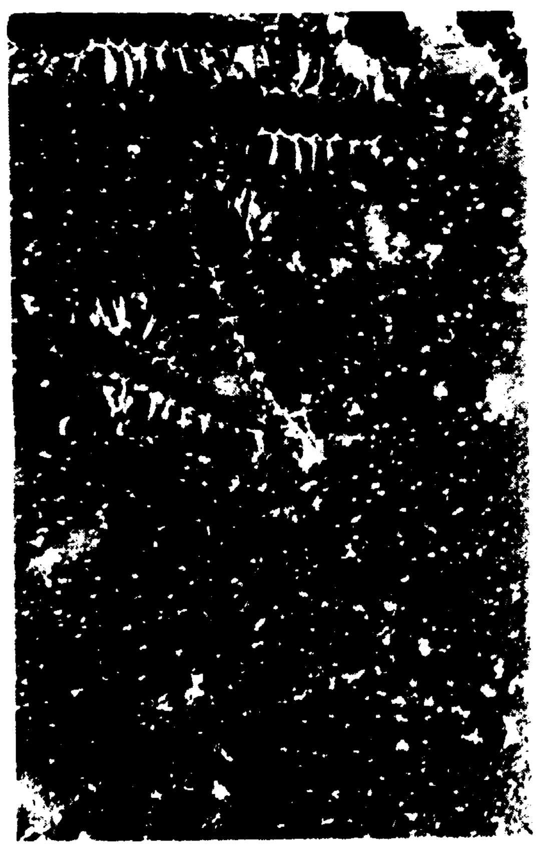

Fig.4, discrete globules of the microbial insecticide being ingested by larval *Anopheles stephensi*
10x

Fig.5, discrete globules of the microbial insecticide being ingested by larval Anopheles stephensi
15x

Fig.6, central figure, empty larval skin of A.stephensi killed by ingestion of formulation 20SSO being eviscerated by a larva of its own species (which commonly practices such necrophagy and presumably thereby eats a concentrate of the bacterial preparation)
15x

It is worthy of comment that the only anopheline routinely colonized at RDC/HIL and used here for efficacy tests of mosquito larvicidal formulations, is Anopheles stephensi. The fact that this species, particularly in urban areas including New Delhi, shows a marked preference for man-made larval habitats (e.g., overhead or ground-level cisterns, barrels, fuel drums, concrete tanks etc.) - shallow to very deep wells being similarly utilized in semi-rural and rural localities - makes it "amenable to very easy laboratory colonization"(see p.416 of Rao,T.Ramachandra, 1984, "The Anophelines of India", Malaria Research Centre, ICMR, xvi, 518pp.). I was informed by Drs Bhateshwar and Ramdev that the decidedly more important malaria vector, A. culicifacies, has proved refractory to colonization here. This is most unfortunate, as it is envisaged that the Bacillus sphaericus formulation discussed and illustrated on pp.5-7 of this Report will eventually be used to a considerable extent against this species, larvae of which, brought in from natural habitats, appear to have fed on the discrete surface globules (pp. 6 & 7) even more readily than does A.stephensi.

While Rao (same ref.as in previous para., pp.326-327) writes that A.culicifacies had "till recently been regarded as not amenable to laboratory colonization in small cages", he provides references from the second half of the 1970s indicating a breakthrough in lab. colony establishment of this species through "modern techniques which were not available to the workers in the earlier years" (such as "a sophisticated system of the switches for providing artificial dawn and dusk light conditions").

In discussions of these matters with Drs Bhateshwar and Ramdev, I was also fully informed on their methodology for testing* e.g. Bacillus sphaericus (1593M) 05SSO efficacy against Anopheles stephensi. Using 50 third instars of the latter, and trays having a surface area of 750 cm^2 and containing 900 ml of tap water, these entomologists demonstrated their procedure to me, using formulation concentrations (mg ai/750 cm^2) of 0.115, 0.230, 0.345, 0.460 and 0.575, plus a blank and a control. The results of the test witnessed (which as is evident showed anomalies from concentrations 0.230 to 0.345, and from 0.460) to 0.575) were as follows.-

CONC. mg ai/750cm^2	% MORTALITY 24 hrs	48 hrs	72 hrs
0.115	1/50 (2%)	1/50 (2%)	2/50 (4%)
0.230	12/50(24%)	19/50(38%)	27/50(54%)
0.345	9/50(18%)	13/50(26%)	28/50(56%)
0.460	13/50(26%)	21/50(42%)	28/50(56%)
0.575	10/50(20%)	14/52(28%)	19/50(38%)
Blank	0/50 (0%)	0/50 (0%)	0/50 (0%)
Control	1/50 (2%)	1/50 (2%)	2/50 (4%)

*The standard formulation 20SSO reportedly gives 100% mortality of A.stephensi. The present demonstration with diluted formulation was to illustrate concentration dependence of this B. sphaericus preparation to mortality of this anopheline.

Limited trials with A.culicifacies larvae derived from another laboratory yielded virtually no mortality over the same range of concentrations of this formulation.

My assignment terminated with a lecture at RDC/HIL reviewing biocides.

II. CONCLUSIONS AND RECOMMENDATIONS

I was impressed by the overall and group leadership exhibited at RDC/HIL, by the enthusiasm and competence of all concerned and their capacity to collaborate towards a common goal, and by the general laboratory and technical facilities at the Gurgaon Centre. While data storage and retrieval facilities could be improved, this need is an almost universal one given the speed of computerization in recent years. My overall assessment is that RDC/HIL, after rapid progress in its laboratory and pilot plant endeavours, is reaching the point where short- and long-term biological field programmes targeted to a short list of prime mosquito vectors of disease could be added to its activities with great advantage. My relevant recommendations are.-

A. "TAILORING" OF MICROBIAL INSECTICIDE FORMULATIONS TO SPECIFIC FIELD PROBLEMS. The health and environmental safety of both Bacillus thuringiensis spp.israelensis and B.sphaericus now being for all practical purposes assured, formulations maximizing the particular advantages of each of these species must be developed. The latter organism combining what seems likely to prove a lesser degree of efficacy against anophelines than the former, with a greater degree of tolerance for heavily polluted waters plus the ability to replicate therein for up to several months, may thus be represented as the better candidate for larviciding against such utilizers of organically rich waters as the filariasis vectors, Culex quinquefasciatus (largely urban) and Mansonia uniformis (semi-rural/rural, in association with Water Hyacinth plants, Eichhornia crassipes).

Slow-release granular formulations of B.sphaericus could prove useful against M.uniformis, and floating maize-based formulations of this bacterium against C.quinquefasciatus.

RDC's 2033SO formulation seems to me to be eminently well suited as a vehicle for applying killed-culture Bacillus thuringiensis ssp. israelensis against Anopheles spp. including A.culicifacies.

Additionally, because of the compatibility of B.thuringiensis ssp.israelensis with the insect growth regulator, methoprene (see ref., 1st para., p.4 of this Report), and the facts that

A. (contd) while this bacterium lacks the residual potentiality of B.sphaericus, methoprene can supply the deficit, slow-release granular formulations incorporating both could have wide applicability against (in particular) a variety of aedine and culicine mosquito pests and vectors — the former including the chief and secondary vectors of dengue haemorrhagic fever, Aedes aegypti and A.albopictus, and the latter, the transmitter of Japanese B encephalitis, Culex tritaeniorhynchus ssp. summorosus. The development and testing of such formulations featuring a blend of two innovative agents would of course call for both laboratory investigations and subsequent short- and long-term field testing. Such an approach would be very much in line with one of the topics to be discussed at the 19-21 October WHO (Special Programme,R.T. Tropical Diseases) "Informal Consultation on Bacterial Formulations for Cost-Effective Vector Control in Endemic Areas" Pondicherry, Vector Control Research Centre, ICMR. - i.e., the short-lived impact of some entomopathogenic bacterial formulations associated with their lack of persistence through failure to remain long enough in the feeding zone of target mosquito larvae.

B. It is considered important that a self-sustaining laboratory colony of Anopheles culicifacies be established in the near future at RDC/HIL (see first two paras, p.8) Towards this end it would be desirable to maintain closer collaboration with other Indian institutions that have made substantial progress in this connection. It might prove productive, too, to attempt to overcome mating problems via the forced copulation technique (an authority on this, who I am sure would be glad to supply his relevant publications and other advice, is: Dr Ivan McDaniel, University of Maine, Orono, ME, USA). It might also prove rewarding to investigate A.culicifacies oviposition stimulants and attractants, via relevant research involving aquatic vegetation and other biological determinants of larval habitats of this species.

C. Such field work should be developed towards comprehensive, long-term studies of the flora and fauna of a small number of carefully selected larval habitats, typical not only of A.culicifacies but also of other locally important disease vectors, e.g. A.stephensi, Culex quinquefasciatus and Aedes aegypti. The extreme taxonomic diversity of most larval mosquito habitats is only now becoming appreciated (see Laird, M., 1988, ref. at end of second para., p.2, of Trip Diary, which includes specifically detailed inventories of the flora and fauna of selected such habitats, amounting to as many as 276 taxa in one instance, and 272 in another). Without preliminary taxonomic research of the kind (calling for the establishment of liaison with appropriate taxonomists in the

C. (contd) various aquatic plant and animal specialties, in India and where necessary, elsewhere), it will not be feasible to comprehend food-web dynamics basic to critical appraisal of another key topic of the forthcoming WHO "Informal Discussions..." at Pondicherry (see first para., p.10, herein).- "The implications of delivery of bacterial toxins through their introduction and expression in other organisms present in the ecological niche of the target larvae are also important and need to be considered even at this early stage."

D. Critical studies of the impact of RDC/HIL microbial insecticide formulations on nontarget organisms (NTOs) as well as on target mosquito larvae, are much to be desired. On the one hand, it already seems unlikely from the results of exhaustive studies elsewhere (see Laird, M., Lacey, L.A., and Davidson, E.W., Eds, in press, ref. in second para., p.4, of Trip Diary) that any aquatic organisms other than mosquitoes themselves and some of their closer dipteran relatives are likely to suffer harm from bacterial insecticide applications. But on the other, it must be remembered that certain important predators on dangerous mosquitoes belong to the same family, Culicidae. These include, in India, species of the small-container utilizing genus Toxorhynchites (the adults of which are not blood-feeders), which are natural control agents against Aedes aegypti; and Culex (Lutzia) spp. The latter seldom bite as adults, and their actively predacious larvae can be important natural population regulatory factors against vectors utilizing various types of ground ponds and pools including Anopheles spp. and Culex quinquefasciatus. Some studies elsewhere have already revealed some adverse impact of Bacillus thuringiensis ssp. israelensis on Toxorhynchites spp., and Indian investigations concerning Culex (Lutzia) spp. are needed in the interests of rational development of future highly selective (i.e. as well as effective against target Culicidae) and fully integrated vector control methodologies; combining source reduction (with appropriate community participation) with the conjoint use of microbial insecticides and insect growth regulators against aquatic stages of mosquitoes, and the continuing use of suitable synthetic chemical control agents as residual adulticides and space-sprays. In this context it must be kept in mind that in late-1988 microbial formulations still comprise only about 1.5% of all insecticides used for the control of insects of agricultural, forestry and medical significance.

Again, while work reviewed in the above forthcoming book on relevant safety issues is reassuring as regards the unlikelihood of harm to man and other vertebrates, special Indian situations merit investigation. For example, water buffaloes very commonly rest half submerged in shallow ponds harbouring anophelines and other mosquito vectors of disease; so is there, then, any risk that their long-continued repetitive

D. (contd) exposure to bacterial insecticides might perhaps lead to harm through entomopathogen entry via the eyes and other body apertures of these semi-aquatic bovids?

E. Although much work in the Americas and West Africa has demonstrated an extremely high degree of safety of Bacillus thuringiensis ssp. israelensis to lotic (flowing water) NTOs in streams and rivers harbouring blackflies (Simuliidae), it will be desirable to undertake similar testing with respect to B.sphaericus formulations as applied in India to vegetated stream margins producing e.g. Anopheles culicifacies; to identify any possible adverse effects of treatments on predators and other NTOs.

F. Finally, in the interests of optimum two-way international collaboration towards the earliest possible commercialization of urgently needed additional formulations of biocides for practical use in safe and effective integrated mosquito control methodologies, it is submitted that much of mutual value could result from present and future RDC/HIL microbial insecticide preparations being made available for further testing elsewhere, for example to evaluate their potentialities against non-Indian Culicidae in e.g. Canada, the USA and Australasia.

III. PERSONS MET BY THE MISSION

1)	Mr. M. Islam	SIDFA, UNDP/UNIDO New Delhi, India
2)	Mr. S.P. Dhua	Chairman & Managing Director, Hindustan Insecticides Ltd
3)	Mr. P.K. Ramdas	Pesticide Development Programme India
4)	Mr. R.K. Khandal	Pesticide Development Programme India
5)	Mr. S.Y. Pandey	Pesticide Development Programme India
6)	Mr. P.K. Patanjali	Pesticide Development Programme India
7)	Mr. N.R. Bhateshwar	Pesticide Development Programme India
8)	Mr. Y.P. Ramdev	Pesticide Development Programme India
9)	Mr. C.P. Batra	National Research Centre
10)	Mr. C.R.K. Pillai	National Reserch Centre