



ICMR BULLETIN



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PROSPECTS OF INCORPORATING BIOLOGICAL AGENTS IN VECTOR CONTROL PROGRAMMES

Vector-borne diseases are major causes of morbidity and mortality in tropical countries. In many instances vector control remains the main, sometimes the only, tool for the prevention/elimination of outbreaks of vector-borne diseases. For several years, chemical pesticides have been used successfully for this purpose. Unfortunately, several species of vector mosquitoes have developed resistance to many pesticides¹. Highly potent but more expensive chemical compounds are being developed as replacements — which may also fail with the emergence of resistance. As a result, the cost of vector control has become very high. Also the Government as well as the public are becoming increasingly concerned about contamination of the environment from the use of chemical insecticides. Nevertheless, chemical insecticides will continue to be used in vector control programmes until effective alternative tools are developed.

The biological control of vectors through regulation of vector proliferation is one of the alternative vector control strategies². In the past, a number of candidates have been studied for vector control potential, including fungi, bacteria, protozoa, nematodes, invertebrate predators and fishes and sufficient information has been gathered with reference to the range of activity of all these organisms as well as their limitations. For various reasons, many of them have proved to be of little practical use and only a few have reached the stage of operational use. Under this category two spore

forming bacteria viz. *Bacillus thuringiensis* var. *israelensis* and *B. sphaericus* can be included.

Bacillus thuringiensis var. *israelensis* produces highly specific toxins against *Anopheles* and *Culex* mosquitoes and blackflies³. Different formulations of *B.t.* var. *israelensis* such as water dispersible powder (WDP), wettable powder (WP), emulsifiable concentrate (EC), flowable concentrate (FC), granules and dust have been used for the control of anophelines effectively⁴. However, the residual activity of these formulations was found to be limited to 24-48 h post-treatment suggesting the need for weekly applications to prevent pupal production. Certain slow release formulations such as briquettes, polypropylene pellets, alginate beads and microgels have been reported to provide significant level of control with residual activity extending to 2 to 8 weeks. The mosquito species tested include, *An. albimanus*, *An. arabiensis*, *An. crucians*, *An. culicifacies*, *An. franciscanus*, *An. freeborni*, *An. gambiae*, *An. punctipennis*, *An. quadrimaculatus*, *An. sinensis*, *An. subpictus*, *An. sudaicus*, *Aedes taeniorhynchus*, *Ae. tarsalis*, *Ae. vexans*, *Cx. nigripalpus*, *Cx. quinquefasciatus* and *Culiseta inornata*.

B.t. var. *israelensis* is widely used against nuisance mosquitoes and blackflies in Europe and North America and is considered as the key larvicide for blackfly (*Simulium damnosum*) control operations in the Onchocerciasis

Control Programme in West Africa⁵. Without *B.t. var. israelensis* it would have been almost impossible to manage resistance to chemical pesticides in the *S. damnosum* complex.

Another spore-forming bacterium, *Bacillus sphaericus* is now considered as the most promising organism for the control of *Culex* mosquitoes⁶. *Culex* species are important vectors of lymphatic filariasis in tropical countries and a nuisance in many temperate countries. As in the case of *B.t. var. israelensis*, application of formulations like WDP, WP, EC, FC, granules and dust causes significant level of control with residual activity limited to 24-48 h only⁴. Use of formulations such as briquettes, polypropylene pellets, alginate beads and microgels resulted in appreciable level of control for 2-8 weeks. The mosquito species tested with these formulations include, *An. gambiae*, *An. subpictus*, *Cx. stigmatosoma*, *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus*, *Mansonia* spp. and *Psorophora columbiae*.

The primary target of currently available formulations of biocontrol agents being aquatic stages of vectors, the existing knowledge of larviciding with chemicals must be taken into account. Being larvicides, the direct effect of these biocontrol agents on disease control is often difficult to demonstrate. So far, only a few reports have correlated the effectiveness of biocontrol agents with adult density and disease control.

Treatment of a dust formulation of *B.t. var. israelensis* over an area of 283 ha of rice fields to control *An. sinensis* at 8 day intervals, resulted in significant reduction in malaria cases⁷. Application of a granular formulation of *B. sphaericus* in rice fields at 15 day interval (10 cycles) to control *An. gambiae*, brought about 13.6 per cent decrease in human biting density⁸. Treatment of rivers, ponds, dams and pits with a liquid formulation of *B. sphaericus* resulted in significant reduction in the number of *Cx. quinquefasciatus* adults landing on human baits⁹. A demonstration project in Cameroon (in a city with 200 hectares of breeding surface) showed that by applying *B. sphaericus* every three months the population of *Cx. quinquefasciatus* female mosquitoes could be brought down by 41-55 per cent¹⁰. A study concluded recently in Mayiladuturai, in Tamilnadu, has shown that by treating the breeding sites of *Cx. quinquefasciatus* with a slow release formulation of *B. sphaericus* at bimonthly interval, a maximum of 41 per cent

reduction in density of man biting adults, 38 per cent reduction in the density of indoor resting adults and 37 per cent reduction in the transmission of bancroftian filariasis could be achieved (VCRC unpublished data).

Several issues should be considered before deciding the strategy for using or incorporating biocides in control operations. Biotic and abiotic factors of breeding sites limit the wide use of selective and specific biocontrol agents. For example, open water bodies without heavy cover of vegetation can be readily treated with the WDP as well as granular formulations. Breeding habitats covered with thick vegetation, however, require the application of granular or floating formulation which can easily penetrate the plant canopy¹¹. Similarly, floating formulations are not suitable for use in large expanses of breeding sources unless they are pegged at different places.

The breeding habitats of *Cx. quinquefasciatus* are generally more polluted and hence the formulations need to be applied at a higher dose for effective control¹² than what is used in less polluted waters as in the case of chemical larvicides¹³. *An. stephensi* mostly breeds in potable water sources like wells and overhead tanks and therefore sufficient caution must be exercised while using formulations of microbial origin. In such situations formulations containing non-viable spores of bacteria or prepared from asporogenic forms¹⁴ can be used as they are unlikely to pose health hazards. The feeding behaviour of aquatic stages of vectors should also be considered. For anopheline targets, floating bait formulations of *B.t. var. israelensis* have been found to be highly effective¹⁵.

If biocontrol is to go beyond the stage of novelty, the general public and the authorities have to be so convinced. In spite of the biosafety of larvicidal formulations of *B.t. var. israelensis* and *B. sphaericus* having been thoroughly verified^{3,6}, environmentalists are sceptical about introducing them into the environment and they must be convinced. Chemical insecticides have been used for more than four decades and biocontrol agents could replace them in certain situations. This is not being done in operational activities as the control managers are unwilling to change the control strategy due to cost considerations. In USA and Germany, *B.t. var. israelensis* is used against non-vector mosquitoes and in those countries cost is not a very important factor. But, from the point of view of developing

countries cost-effectiveness of any technology is an important factor.

The cost-effectiveness of using biocontrol agents for vector control has been worked out in only a few studies (Table I). A study conducted in Tanzania indicates that it costs US \$ 738 (US \$ 14760/ha) for one year's control of *Cx. quinquefasciatus* in a breeding area of 0.05 ha with *B. sphaericus* as against a cost of US \$ 1188 (US \$ 23760/ha) if chlorpyrifos is used¹⁶. Another report states that the cost of larval control in unpolluted breeding grounds using briquette formulations of *B.t. var. israelensis* and *B. sphaericus* is more or less comparable to the cost of control if fenthion is used¹⁷. In yet another study (VCRC unpublished data)

Table 1. Cost of control of *Culex quinquefasciatus*

Particulars	<i>B. sphaericus</i>	Chlorpyrifos
Studies conducted in Tanzania¹⁶		
Conditions		
Formulation	BSP-1 (12%)	EC (480 g/l)
Dosage	12 kg/ha	4.8 kg/ha
Frequency of treatment	9 rounds/year	24 rounds/year
Area of breeding surface	0.05 ha	0.05 ha
Cost of control		
Staff and equipment	US \$ 378	US \$ 1008
Larvicide	US \$ 360	US \$ 180
Total	US \$ 738 (US \$ 14760/ha)	US \$ 1188 (US \$ 23760/ha)
Studies conducted in India (VCRC's unpublished data)		
Conditions		
Formulation	Spherifix (95%)	
Dosage	15 kg/ha	
Frequency of treatment	6 rounds/year	
Area of breeding surface	0.62 ha	
Cost of control		
Staff and equipment	Rs.1680	
Larvicide	Rs.7500	
Total	Rs.9180 (Rs.14807/ha)	

Superscript no. refers to the sl.no. in the reference list.
VCRC - Vector Control Research Centre, Pondicherry
EC - Emulsifiable concentrate

it has been estimated that the cost of control of *Cx. quinquefasciatus* in a breeding area of 0.62 ha for a period of one year with a slow release formulation of *B. sphaericus* is Rs.9180 (Rs.14807/ha).

Recently, the development of resistance to *B. sphaericus* has been observed in a field population of *Cx. quinquefasciatus*¹⁸. However, there are definite indications from data from studies undertaken so far that the biocontrol agents like *B. thuringiensis var. israelensis* and *B. sphaericus* do have the potential to be incorporated in certain situations of operational programmes. Some of the breeding habitats of vectors where the formulations of the two bacterial agents could possibly be used^{11,12,19-22} are given in the Table II.

Table II. Major breeding habitats of mosquito vectors where *B. thuringiensis var. israelensis* and/or *B. sphaericus* could be used.

Area	Disease	Vector	Nature of breeding source
Urban/rural areas	Bancroftian filariasis	<i>Culex quinquefasciatus</i>	Cess pits, cess pools, drains, disused wells
Urban areas	Malaria	<i>Anopheles stephensi</i>	Overhead tanks and wells
Rural areas	Japanese encephalitis	<i>Culex vishnui</i> gr.	Transient pools
Coastal areas and islands eg Tamilnadu	Malaria	<i>Anopheles culicifacies</i>	Water-holes in coconut and casuarina plantations
Coastal areas eg Kerala	Malayan filariasis	<i>Mansonia</i> spp.	Weed infested ponds and canals

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This write-up is contributed by Dr. K. Balaraman, Deputy Director, Dr. K. Gunasekaran and Dr. S.L. Hoti, Senior Research Officers, Vector Control Research Centre, Pondicherry.

ABSTRACTS

Some Research Projects Completed Recently

Clinicopathologic features and prognostic significance of tumour markers in tongue cancer.

A prospective study was carried out on 90 patients with squamous cell carcinoma of the tongue and 5 with leukoplakia, and 5 autopsy specimens (as controls) to evaluate the prognostic significance of various tumour markers

in tongue cancer. The determinants studied were prolactin (PRL) and bioactive prolactin in blood samples and prolactin mRNA, cathepsin D mRNA and membrane bound epidermal growth factor receptor (EGFR) in primary tumours.

Hyperprolactinaemia was observed in 31 of the 90 (34%) patients with advanced tongue cancer. PRL levels

were significantly higher in tobacco chewers/smokers. PRL levels decreased in patients who responded to various treatment modalities compared to pretherapeutic levels. On the other hand it increased with disease progression. Estimation of bioactive PRL using Nb₂ lymphoma cell lines revealed that of the 90 patients, good correlation between Nb₂ bioassay and immunoradiometric assay was observed in 80 per cent patients with hyperprolactinaemia. The bioactive PRL levels were significantly lower in 50 per cent patients than the immunoradiometric PRL levels.

All the 5 (100%) autopsied tongue specimens showed the presence of cellular RNA while this was seen only in 3 of 5 (60%) patients with precancerous lesions and 40 of the 90 (44%) patients with tongue cancer. PRL mRNA was detected in only 10 of 40 (25%) patients with tongue carcinomas but in none of the autopsied tongue specimens or patients with precancerous lesion. Cathepsin D mRNA was expressed in all 5 (100%) autopsied tongue specimens, none of the patients with precancerous lesions and all 40 patients (100%) with tongue carcinomas who were studied.

Out of 90 patients, 83 (92%) had EGFR positive tumours and 7 (8%) EGFR negative tumours.

It is thus concluded that presence of PRL mRNA confirms its ectopic production by tongue tumours. This ectopically produced prolactin might be acting as one of the major growth promoters via autocrine/paracrine mechanism. Immunoreactive prolactin is a better marker than bioactive prolactin for monitoring disease course. High levels of EGFR is an unfavourable prognosis. Overexpression of cathepsin D mRNA is probably an indicator of tumour aggressiveness. Hyperprolactinaemia clearly differentiates patients with aggressive tumours.

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Effect of X-irradiation on structural and trace element content in integumentary system.

The study was carried out on CF strain albino rats to determine the histological and histochemical changes in the integumentary system and estimate the changes in trace element contents in tissues caused by chronic low dose and acute exposure to X-irradiation. An attempt was also made to determine the occupational radiation hazards to

radiographers. For chronic exposure rats were exposed to chronic intermittent low dose X-radiation at the dose rate of 0.015 cGy/sec/day (7 days irradiation alternating with 7 days rest) for periods of 90, 180, 270, 360, 540 and 720 days. For acute effect the rats were exposed to 7Gy X-radiation (1.211 Gy/minute).

Structural (transmission electron microscopy, scanning electron microscopy and light microscopy) and trace metal (atomic absorption spectrometry) studies revealed that chronic low dose X-radiation affected the structural integrity of the skin and induced imbalance in the trace metal homeostasis of the integumentary system. Transmission electron microscopy revealed increase in apoptosis in the epidermal cells and giant fibrocytes and hyalinization of collagen which started after 6 months of irradiation and became extensive after 24 months. Atomic absorption spectrometry revealed depletion in magnesium and zinc levels and zinc to iron ratio and increase in iron and copper concentrations.

Acute exposure study reconfirmed the damaging effects of chronic low dose exposure to X-irradiation. However, the trace metals did not show any definite pattern of change in their tissue concentrations over the post irradiation period.

Study of human scalp hair and nail samples of medical radiographers and non-radiographers (controls) matched for age and food habits, revealed increase in zinc, copper and iron concentrations along with varied types of cuticular damage in the radiographers compared to non-radiographers. These findings possibly confirm the hazardous effects of long-term exposure to X-rays. It also indicate that the integumentary system (scalp hair and nails) may act as an useful biogenic indicator of radiation hazard.

A.K. Das
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Publications:

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2. Chatterjee, J., De, K., Basu, S.K. and Das, A.K. Collagen, zinc and iron contents of rat skin irradiated with chronic low dose X-ray. *Indian J Med Res* 98: 243, 1993.

ICMR NEWS

Shri A.R. Antule, Hon. Union Minister for Health and Family Welfare presented the ICMR Awards and Prizes to Outstanding Biomedical Scientists on September 30, 1995 at the Vigyan Bhawan, New Delhi.

The following meetings of various technical groups/committees of the Council were held:

Meetings of the Expert Groups (EGs)/Task Forces (TFs)/Project Review Committees (PRCs) held at New Delhi:

TF on Venoms and Toxins	September 19, 1995
TF on Monitoring of Adverse Drug Reactions in India	September 20, 1995
TF on Hospital-based Case — Control Study on Long-term Health Consequences of Vasectomy	September 20, 1995
EG on Traditional Remedies for Cancer	September 20, 1995
EG on Traditional Medicine Research	September 20, 1995
TF on Quality of Community Life	September 21-22, 1995
PRC on HIV Infection	September 25, 1995
PRC on Microbiology and Sexually Transmitted Diseases	September 26, 1995

Participation of ICMR Scientists in Scientific Events:

Dr. U.M. Donde, Dy. Director, Institute for Research in Reproduction (IRR), Bombay, participated in the

VII Asia-Pacific Congress of Clinical Biochemistry at Bangkok (September 17-22, 1995).

Dr. V. Kumaraswami, Assistant Director, Tuberculosis Research Centre, Madras, participated in the meeting of the Task Force for Filariasis Field Trials of the WHO Special Programme for Research and Training in Tropical Diseases at Geneva (September 18-21, 1995).

Dr. V.P. Sharma, Director, and Dr. R.S. Yadav, Senior Research Officer, Malaria Research Centre, Delhi, participated in the Annual Malaria Meeting of the British Society of Parasitology at London (September 19-22, 1995).

Dr. G.V. Satyavati, Director-General, ICMR, delivered the inaugural address at a Symposium on Women and Health at Bangalore (October 1, 1995). She also delivered the inaugural address at the I International Conference on Viral Hepatitis E Virus at New Delhi (October 13, 1995).

Shri Byomkesh Mannna, Research Officer, National Institute of Cholera and Enteric Disease, Calcutta, participated in the International Workshop in Research Methodology at Dhaka (October 8-19, 1995).

Dr. Ikram Khatkhatay, Research Officer, IRR, Bombay, participated in Immunoanalysis — A European Symposium at Lille, France (October 9-13, 1995).

Dr. B.N. Saxena, Senior Deputy Director-General, ICMR, participated in a Symposium on Reproductive Health: A Human Right and a Pillar of Human Dignity at Szeged, Hungary (October 9-19, 1995).

Dr. Kalyan Banerjee, Director, National Institute of Virology, Pune, participated in the Regional Consultation on Strategy for Prevention and Control of Dengue/Dengue Haemorrhagic Fever at New Delhi (October 10-13, 1995).

Dr. R. Prabhakar, relinquished charge as Director, Tuberculosis Research Centre, Madras w.e.f. September 30, 1995.

Dr. C.R. Ramachandran, relinquished charge as Chief, Division of Non-communicable Diseases, ICMR Headquarters, New Delhi, w.e.f. September 30, 1995.

ICMR AIDED SYMPOSIA/SEMINARS/WORKSHOPS/COURSES/CONFERENCES

Symposium/Seminar/Workshop/ Course/Conference	Date & Place	Contact Address
Symposium on Prevention and Control of Goitre Endemism in Tribal Population through Education and Mass Communication.	October 2-3, 1995: (at Bhopal)	Dr. D.K. Belsare, Director, Institute for Natural Resources and Human Environment, Bhopal.
Cytology Update - 1995.	October 14-15, 1995; (at Chandigarh)	Dr. A. Rajwanshi, Organising Secretary of Update, Department of Cytology and Gynaecologic Pathology, Postgraduate Institute of Medical Education and Research, Chandigarh.
XXVI Annual Conference of the Endocrine Society of India.	December 1-3, 1995; (at Ahmedabad)	Dr. J.M. Bhatawdekar, Organising Secretary, ESICON-1995, Division of Molecular Endocrinology, The Gujarat Cancer Society, Ahmedabad.
International Symposium on Radiomodifiers in Human Health.	December 28-31, 1995; (at Manipal)	Prof. P. Uma Devi, Convenor, ISRHH, Department of Radiobiology, Kasturba Medical College, Manipal.

COUNCIL'S TRAINING PROGRAMMES

Reproductive Biology

At the Institute for Research in Reproduction, Bombay:

- Training Course on Techniques in Immunology, Cell Biology and Molecular Biology (November 5-25, 1995).
- Training Course on Techniques in Neuroendocrine Research (February 6-10, 1996).

Nutrition

At the National Institute of Nutrition, Hyderabad:

- Annual Training Course in Nutrition (December 1, 1995 - February 28, 1996).

Occupational Health

At the National Institute of Occupational Health, Ahmedabad:

- Orientation Course on Occupational Health for Industrial Medical Officers (November 13-24, 1995).
- Training Course on Air Pollution Monitoring and Risk Assessment (December 13-19, 1995).

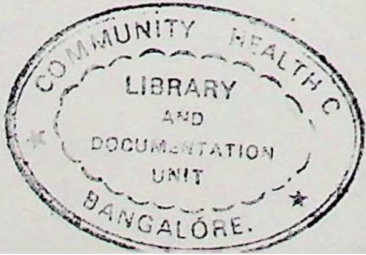
Laboratory Animal Technology

At the Laboratory Animal Information Services Centre, National Institute of Nutrition, Hyderabad:

- Training Course for Laboratory Animal Supervisors (September 4 - November 30, 1995).

ICMR PUBLICATIONS

	Price (Rs.)
Nutritive Value of Indian Foods (1985), by C. Gopalan, B.V. Ramasastri and S.C. Balasubramaniam, Revised and Updated (1989), by B.S. Narasinga Rao, K.C. Pant and Y.G. Deosthale (Reprinted 1995)	25.00
Growth & Physical Development of Indian Infants and Children (1972, Reprinted 1989)	10.00
Studies on Weaning & Supplementary Foods (1974, Reprinted 1986)	6.00



Studies on Pre-School Children (1974, Reprinted 1984)	6.00
A Manual of Nutrition (Second Edition 1974, Reprinted 1992)	4.50
Low Cost Nutritious Supplements (Second Edition 1975, Reprinted 1994)	4.00
Menus for Low Cost Balanced Diets and School Lunch Programmes Suitable for South India (Third Edition 1977, Reprinted 1991)	4.50
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Some Common Indian Recipes and their Nutritive Value (Fourth Edition 1977, Reprinted 1991) by Swaran Pasricha & L.M. Rebello	10.00
Nutrition for Mother & Child (Third Edition 1978, Reprinted 1991) by P.S. Venkatachalam & L.M. Rebello	9.00
Japanese Encephalitis in India (Revised Edition 1980)	5.00
Some Therapeutic Diets (Fourth Edition 1988, Reprinted 1995) by Swaran Pasricha	4.50
Nutrient Requirements & Recommended Dietary Allowances for Indians (1990, Reprinted 1994)	14.00
Fruits (1983, Reprinted 1992) by Indira Gopalan & M. Mohan Ram	7.00
Count What You Eat (1989, Reprinted 1991) by Swaran Pasricha	9.00
Diet & Diabetes (Second Edition 1993) by T.C. Raghuram, Swaran Pasricha & R.D. Sharma	18.00
Dietary Tips for the Elderly (1992, by Swaran Pasricha & B.V.S. Thimmayamma	3.50
Diet and Heart Disease (1994) by Ghafoorunissa and Kamala Krishnaswamy	26.00
*Depressive Disease (1986) by A. Venkoba Rao	58.00
**Medicinal Plants of India Vol.2 (1987)	136.00
**The Anophelines of India (Revised Edition 1984) by T. Ramachandra Rao	150.00

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