

BULLETIN

Of

Haffkine Institute



Haffkine Institute for Training, Research and Testing
Parel, Bombay - 400 012, India

EDITORIAL BOARD

Chairman & Editor

Dr. V. R. DESHPANDE, M.D.

Director, Haffkine Institute for Training, Research and Testing, Bombay

Assistant Editor

Mr. R. B. GHOOI, M. Sc.

Scientific Secretary, Haffkine Institute for Training, Research and Testing
Bombay

Members

Dr. C. G. PANDIT, M.B., B.S., Ph. D., D.P.H., D.T.M.

Ex-Director General, Indian Council of Medical Research

Dr. H. I. JHALA, M.D., F.C.P.S., D.T.M.

Superintendent, Sir Harkisondas N. Hospital, Prarthana Samaj, Bombay

Dr. K. G. NAIR, S.E.M., M.D., Ph.D. (Chicago), F.A.C.C. (U.S.A.)

F.I.C.A. (U.S.A.), F.P.C.C. (Hon.)

Director-Professor of Medicine (Retd.) Seth G. S. Medical College, Bombay

Dr. D. D. BANKER, M.D., Ph.D.

Chief Bacteriologist (Retd.), Glaxo Laboratories, Bombay

Dr. F. FERNANDES, M. Sc., Ph.D.

Head of Microbiology Department (Retd.), Ciba Research Centre, Bombay

Dr. S. S. RAO, M. Sc., Ph.D.

Assistant Director (Retd.), Haffkine Institute, Bombay

Mr. PERCY E. D'SOUZA

Managing Editor, 'Medical Times', Sandoz (India) Ltd., Bombay

Dr. C. V. DELIWALA, M. Sc., Ph.D.

Deputy Director (Retd.), Haffkine Institute, Bombay

Dr. M. V. SANT, M.D., F.C.P.S., D.E. (Prague)

Deputy Director (Retd.), Haffkine Institute, Bombay

Dr. A. N. D. NANAVATI, M.D.

Assistant Director (Retd.), Haffkine Institute, Bombay

Dr. M. B. BHIDE, M.D., F.C.A.I.

Assistant Director, (Retd.), Haffkine Institute for
Training, Research and Testing, Bombay

Dr. F. L. SALDANHA, M. Sc., Ph. D., F.I.C. (Cal.)

Deputy Manager (Quality Control), Haffkine Bio-Pharmaceutical Corporation
Ltd., Bombay

Bulletin of Haffkine Institute

Vol. 11 No. 1

C O N T E N T S

Title & Author	Page
<i>Immunity in Salmonellosis</i> R. S Kamat and Vineeta Bal	1
<i>Comparison of the Hypoglycemic effects of three different extracts from Bordetella pertussis</i> M. Kishore and P. Ramakrishnan	7
<i>Food habits of the lesser Bandicoot Rat</i> D. M. Renapurkar and P. J. Deoras	11
<i>Binding of Monoene Bhilawanol on Clostridium tetani</i> B. K. Patwardhan, U. R. Phadke and R. B. Ghooi	14
<i>Individual and synengetic activity of some essential oils as Mosquito Larvicidal Agents</i> S. R. Chavan, Neena P. Shah and S. T. Nikam	18

FORM IV
(See Rule 8)

1. Place of Publication : Haffkine Institute for Training
Research and Testing,
Parel, Bombay - 400 012.

2. Periodicity of its Publication : every 4 months

3. Printer's Name : Dr. V. R. Deshpande
Whether citizen of India ? : Indian
(If foreigner state the country of origin) : Nil
Address : Haffkine Institute for Training
Research and Testing,
Parel, Bombay - 400 012.

4. Publishers' Name : Dr. V. R. Deshpande
Whether citizen of India ? : Indian
(If foreigner state the country of origin) : Nil

5. Editor's Name : Dr. V. R. Deshpande
Whether citizen of India ? : Indian
(If foreigner state the country of origin) : Nil
Address : Haffkine Institute for Training
Research and Testing,
Parel, Bombay - 400 012.

I, Dr. V. R. Deshpande, hereby declare that the particulars given above are true to the best of my knowledge and belief.

Date : 1-3-1983

Sd/-
(Dr. V. R. Deshpande)

Immunity in Salmonellosis

R. S. KAMAT and VINEETA BAL

Department of Clinical Pathology

Haffkine Institute, Parel, Bombay 400 012.

Salmonella are responsible for a wide spectrum of human diseases. Human salmonellosis manifests in one of the following common forms namely enteric fever, enterocolitis, bacteraemia, localised inflammatory states such as arthritis, meningitis, osteomyelitis and lastly chronic carrier state. Enteric fever is usually caused by *S. typhi*, *S. paratyphi A, B* and *C*, the last organism being not commonly encountered in India.² *Salmonella* enterocolitis as a cause of sporadic diarrhoea is not very common. In a recently conducted study in our laboratory, on 275 cases of sporadic diarrhoea, in 8.0% of cases *Salmonella* were isolated.³ Even though this indicates *Salmonella* to be an uncommon cause of sporadic diarrhoea the disease itself being extremely common, the frequency is not so low as to be ignored as insignificant. Similarly judging from the fairly high incidence of the presence of anti-flagellar antibodies in the asymptomatic individuals, the incidence of infection, due to enteric group of organisms also appears to be quite significant.

Study of immune mechanisms against *Salmonella* is of great interest for many reasons. First of all, the current oral typhoid vaccines, though to an extent effective⁴ are far from satisfactory. The disease though of a systemic nature, has no long-lasting immunity. The

relapse and the carrier state are not very infrequent.⁵ One would like to know the status of immunity in these situations. All the 2000 and odd species¹ which are frequently encountered in the environment may modify the immune status of the host. *Salmonella* enterocolitis may also alter the immune state of the host towards enteric group of organisms. In view of these, a review of the basic immune mechanisms operative in salmonellosis seems very relevant.

Local Immunity in the Gastrointestinal Tract

Theoretically antibacterial antibodies in the intestinal secretions should be able to offer protection against infections provided the antibodies are directed towards the relevant antigens of *Salmonella* participating in adhesion and penetration of the mucus membrane. Hitherto such antigens have not been identified. It is important to point out here that the live salmonellae do possess important properties necessary for induction of secretory immune response.⁶ As pointed out by Pierce and Gowans⁷ the immunogen must stimulate Peyer's patch B lymphocytes and probably T lymphocytes of the mesenteric lymph nodes for a good secretory antibody response. It is possible for an invasive organism such as *Salmo-*

nella to deliver its relevant antigen to the important lymphoid tissue. Success of a live attenuated vaccine would essentially depend upon the ability of the strain to achieve this. Nevertheless the issues such as sustenance and anamnestic response in the gastrointestinal tract are far from settled. Of prime importance in the oral vaccine are retention of relevant antigens and invasiveness. In order that the new vaccines now being developed are examined critically, one needs to identify the antigens involved in adhesion and penetration of the mucus membrane.

Systemic Immunity — Salmonella can survive within neutrophils and normal macrophages of nonimmune host⁸. However, they can be effectively destroyed in an immune host by macrophages known, as 'activated macrophages', generated by the interaction of effector T cell, antigen and macrophage⁹. This interaction between T cell and the macrophages is of dual nature, both the antigen and the products of I region of the Major Histocompatibility Complex participating in the interaction¹⁰. This obviously necessitates display of the relevant bacterial antigen on the macrophage membrane in association with the I region product. 'Activated macrophages' so generated are nonspecific in their spectrum and can kill a totally unrelated organism¹¹. Controlled experiments have given a strong indication that the humoral immunity is unable to offer protection^{11, 12, 13}. In spite of this there are two schools of thought one led by Jenkins and Rowley and the other by

Mackaness and his associates, over the relative importance of humoral and cell mediated immunity (CMI) in salmonellosis. The former claim that the 'activated macrophages' function by virtue of the cytophilic antibody present on the macrophage membrane¹⁴. However, Mackaness and his co-workers^{11, 13, 15} have clearly shown an inherent change in the macrophage on activation. The activated macrophage is nonspecific in its killing potential, which would not be so had its function been due to the cytophilic antibody. Nevertheless some experimental data need to be clarified. Khedkar et al¹⁶ have clearly shown that the mice immunised with formalised vaccine, having a good antibody response and a poor or no cell mediated immune response, can, definitely eliminate challenge salmonellae from their tissues more efficiently than normal nonimmune mice, though not as efficiently as mice immunised with live *Salmonella* and having a good CMI. Again, clinical trials with killed vaccines which generate only an antibody response do show a definite protection (^{17, 18, 19, 20}). These data do not necessarily rule out importance of cellular immunity. It is possible that the outbred mice grown in a non-germ-free environment possess activated macrophages in reasonable numbers which can be very effectively focussed on the challenge salmonellae by the antibody. Same may be true in human beings immunised with killed vaccine.

Nair and Kamat²¹ have shown that the activated macrophages are short lived and their numbers decline follow-

ing recovery from infection. Since killed vaccines can not stimulate CMI to generate activated macrophages, it is important to know the trigger signals required to generate them. The effector T cells are known to be long lived and by making use of this property it was shown by the above investigators that the activated macrophages can be generated by sonic extract of *Salmonella* without having to resort to fresh immunisation²¹. This important finding suggests another approach for development of new vaccine, namely a live attenuated vaccine to be used for systemic immunisation.

Target Antigens of Cell Mediated Immunity — Since CMI is so important in salmonellosis it is essential to identify antigens participating in these responses. This knowledge will enable a logical investigation of immune status in chronic carrier states and in cases of unusual presentation of *Salmonella* infection. Known antigenic relationship amongst *Salmonella* as per the Kauffmann and White scheme is based on somatic antigen or lipopolysaccharide (LPS) and flagellar antigens in both phases²². The scheme may have no relevance with reference to CMI, if the antigens involved in the response are neither the LPS nor the flagellar antigens. This information is of extreme importance because a large number of species of *Salmonella* with varying degrees of virulence to man, that are present in our environment may modify the immune status of the human host. Nair and Kamat²¹ have studied cross reactions between some *Salmonella* employ-

ing CMI as the test parameter. In their study the delayed hypersensitivity (DH) responses could be elicited in *S. enteritidis* (Group D) immunised mice by the sonicate of the immunising strain *S. typhi* and *S. gallinarum* (both group D). Since the last organism is an aflagellate, it is likely that the flagellar antigens do not participate in CMI. The DH responses could be elicited in these mice by the sonicate of *S. paratyphi C* (Group C) also. The sonicate of the same organism was even capable of generating 'activated macrophages' in *S. enteritidis* immunised mice. These data clearly indicate that the antigens involved in CMI are likely to be other than LPS and flagellar antigens. Padmanaban and Mittal²³ have similarly reported cross-protection between salmonella belonging to different serogroups.

Environmental Salmonella and Immunity :

The genus *Salmonella* consists of 2000 and odd species, not all of which are highly pathogenic to man. Hence, sub-clinical infections and the concomitant modulation of the immune status are likely events. If a number of these species are related to human pathogens, particularly the enteric group of organisms with respect to antigens relevant in CMI, its status in the human host who has undergone such a subclinical infection would ultimately decide his resistance to the disease. Such infection need not necessarily enhance the resistance. Antigens administered by oral route are known to induce a state of immune suppression²⁴. A subclinical or a mild *Salmonella* enterocolitis may bring

about such immune deviation resulting into increased susceptibility.

Vaccines —

Having reviewed the mechanisms of immunity it would be interesting to survey the different vaccines so far used.

It is the general experience that the killed vaccines do not induce CMI (¹¹, ¹³, ²⁵). However mice immunised with killed vaccine do show enhanced resistance to challenge infection (²⁰, ²⁶). Immunisation of mice with heat-killed, alcohol-killed, formalised or acetone-killed vaccines can enable the mice to withstand a challenge from 10-100 LD 50 depending upon the dose of the vaccine, interval between immunisation and challenge, the mode of inactivation and the virulence of the challenge strain (²³, ²⁷, ²⁸, ²⁹). Jenkins and Rowley¹⁰ observed the following order of protection in mice immunised with live cells or 250 μ g of killed cells prepared in different ways : when challenged with 1000 LD 50, live cell immunised group showed 90% survival, acetone and alcohol killed cell immunised group 70-80% survival and heat-killed cell immunised group only 10% survival. It appears that the killed bacterial vaccines induce definite protection in experimental animals without generating CMI. The possible mechanism of protection has already been alluded to earlier. Khedkar et al.¹⁶ have also ruled out the possibility that the anti-LPS

antibodies generated by killed vaccines owed their protective function to the neutralisation of the bacterial endotoxin.

Clinical trials with killed vaccines prepared in different ways certainly show some protection (¹⁷, ¹⁸, ¹⁹, ²⁰). However the evidence to support the importance of preservation of Vi antigen in the vaccine is far from convincing (¹⁷, ¹⁸, ²⁶). Since in a clinical situation an individual does not have to face a challenge with virulent salmonellae in doses that are multiples of LD 50, in the absence of a better vaccine, different forms of inactivated vaccines have been in use for a long time.

Oral inactivated vaccines have been ineffective in field trials³¹. Killed bacterial suspensions given orally do not induce sustained antibody response in immunised animals. In a recent trial conducted in Egypt⁴ where enteric fever is endemic, a new oral live attenuated vaccine prepared from a galactose epimerase mutant strain of *S. typhi* (Ty 21 a) was found to protect significantly the immunised population. The vaccine was found to be safe, stable and effective for at least one year. Whether this vaccine would protect against the other organisms causing enteric fever remains to be seen. Since this is an oral vaccine it is essential to investigate the potential of this vaccine to bring about suppression of CMI.

REFERENCES

- 1 Wilson, G. S. and Miles, A.A. (1975) — Topley and Wilson's Principles of bacteriology, virology and immunity. Edward Arnold. p. 933 p. 919.
- 2 Ananthanarayan, R. and Jayaram Paniker, C. K. (1981) — Textbook of microbiology. Second edition, Orient Longman, New Delhi, p. 277.
- 3 Mirchandani, R. M. (1981) — Serotypes and pilus antigens of enterotoxigenic *Escherichia coli*. A thesis submitted to the University of Bombay for the degree of Master of Science.
- 4 Wahdan, M. H.; Serie, Ch.; Germanier, R.; Lackany, A.; Cerisier, Y.; Guerin, N.; Sallam, S.; Geoffroy, P.; Saderk-El-Tantawi, A. and Guesry, P. (1980) — A controlled field trial of live oral typhoid vaccine Ty 21a. **Bull. W.H.O.** 58, 469.
- 5 Wilson G. S. and Miles A. A. (1975) — Topley and Wilson's Principles of bacteriology, virology and immunity. Edward Arnold p. 2009.
- 6 Hohmann, A.; Schmidt, G. and Rowley D. (1979) — Intestinal and serum antibody responses in mice after oral immunisation with *Salmonella*, *Escherichia Coli* and *Salmonella-Escherichia Coli* hybrid strains. **Infect. Immun.** 25, 27.
- 7 Pierce, N. F. and Gowans, J. L. (1975) — Cellular kinetics of the intestinal immune response to cholera toxoid in rats. **J. Exp. Med.** 142, 1550.
- 8 Suter, E. (1956) — Interaction between phagocytes and pathogenic microorganisms. **Bacteriol. Rev.** 20, 94.
9. Mackaness, G. B. (1971) Resistance to intracellular infection **J. Inf. Dis.** 123, 439.
- 10 Zinkernagel, R. M.; Callahan, G. N.; Althage, A.; Cooper, S.; Klein, P. A. and Klein, J. (1978) — On the thymus in the differentiation of 'H-2 self-recognition' by T cells: Evidence for dual recognition? **J. Exp. Med.** 147, 882.
- 11 Blanden, R. V.; Mackaness, G. B. and Collins F. M. (1966) Mechanisms of acquired resistance in mouse typhoid **J. Exp. Med.** 124, 585.
- 12 Collins, F. M. (1970) — Immunity to enteric fever in mice. **Infect. Immun.** 1, 243.
- 13 Mackaness, G. B.; Blanden, R. V. and Collins, F. M. (1966) — Host-parasite relations in mouse typhoid. **J. Exp. Med.** 124, 573.
- 14 Jenkins, C. R. and Rowley, D. (1963) — Basis for immunity to typhoid in mice and the question of 'cellular immunity' **Bacteriol. Rev.** 27, 391.
- 15 Collins, F. M.; Mackaness, G. B. and Blanden, B. V. (1966) — Infection — immunity in experimental salmonellosis. **J. Exp. Med.** 124, 601.
- 16 Khedkar, S.; Sharma, K. D. and Kamat, R. S. (1982) — Relative importance of humoral and cellular immune response in experimental *Senteritidis* infection. **Indian J. Med. Res.** 76, 65.
- 17 Yugoslav typhoid commission (1962) — A controlled field trial of the effectiveness of phenol and alcohol typhoid vaccines. **Bull. W.H.O.** 26, 357.
- 18 Yugoslav typhoid commission (1964) — A controlled field trial of the effectiveness of acetone-dried and inactivated and heat-inactivated typhoid vaccines in Yugoslavia. **Bull. W.H.O.** 30, 623.
- 19 Typhoid pannel, U. K.; Department of technical co-operation (1964) — A controlled field trial of acetone-dried and inactivated and heat-phenol inactivated typhoid vaccines in British Guiana. **Bull. W.H.O.** 30, 631.

- 20 Polish typhoid committee (1966) — Controlled field trials and laboratory studies on the effectiveness of typhoid vaccine in Poland. 1961-64. **Bull. W.H.O.** 34, 211.
- 21 Nair R. and Kamat, R. S. (1982) — Effector cell mediated immune response in mice immunised with *Salmonella*, **J. Med. Microbiol.** 15, 215.
- 22 Edwards, P. R. and Ewing, W. H. (1972) Antigenic scheme for *Salmonella* in 'Identification of Enterobacteriaceae' 3rd ed, Burgess Publ. Co.; Minneapolis. p. 208.
- 23 Padmanaban, V. D. and Mittal K. R. (1979) — Cross protection against *Salmonella enteritidis* infection in mice: I: immunisation trials **Acta Microbiol. Acad. Sci. Hung.** 26, 277.
- 24 Rees, R. and Cebra, J. (1975) — Anti dinitrophenyl antibody production in strain 13, guinea pigs fed or sensitised with dinitrochlorobenzene. **J. Immunol.** 18, 723.
- 25 Ushiba, D. (1965) — Two types of immunity in experimental typhoid 'Cellular immunity' and 'humoral immunity' **Keio J. Med.** 14, 45.
- 26 Hejfec, L.; Salim, L.; Letjman M., Kuz 'minova' M.; Vasil'eva, A.; Levina L.; Bencianova T.; Palova E. and Antonova A. (1966) — A controlled field trial and laboratory study of five typhoid vaccines in the U.S.S.R., **Bull. W.H.O.** 34, 321.
- 27 Angerman, C. and Eisenstein, T. (1978) — Comparative efficacy and toxicity of a ribosomal vaccine, acetone killed cells, LPS and a live cell vaccine prepared from *S. typhimurium*. **Infect. Immun.** 19, 575.
- 28 Collins, F. M. (1968) — Cross protection against *S. enteritidis* infection in mice. **J. Bacteriol** 95, 1343.
- 29 Collins, F. M. (1968) — Recall of immunity in mice vaccinated with *S. enteritidis* or *S. typhimurium*. **J. Bacteriol** 95, 2014.
- 30 Jenkins, C. R. and Rowley, D. (1965) — Partial purification of the 'protective' antigen of *S. typhimurium* and its distribution amongst various strains of bacteria'. **Aust. J. Exp. Biol. Med. Sci.** 43, 65.
- 31 Chuttani, C. S.; Prakash, K.; Gupta, P., Grover, V and Kumar, A. — Controlled field trial of a high-dose oral killed typhoid vaccine in India, **Bull. W.H.O.** 55, 645.

Comparison of the Hypoglycemic Effects of Three Different Extracts from *Bordetella pertussis*

M. KISHORE and P. RAMAKRISHNAN.

Department of Biochemistry, Haffkine Institute, Parel, Bombay 400 012.

The hypoglycemic (blood sugar lowering) factor shown to be present in *Bordetella pertussis* cells or extracts, has not been purified so far. The main problem appears to be the extraction of this factor, apart from other biological factors present in *B. pertussis*, in an aqueous soluble form. In the present work three different methods of extraction which yield aqueous soluble preparations of *B. pertussis*, have been attempted, and their hypoglycemic effects compared in Haffkine strain mice. The three extracts, as compared to controls, show significant lowering of blood glucose levels (13-25% lowering). No significant difference, however, is observed in the hypoglycemic effect between the three individual preparations. The extraction method employing enzymes, being relatively free from contaminants, is used for further purification and characterization of the hypoglycemic factor from *B. pertussis*.

Introduction :

Bordetella pertussis cells contain a substance which, when injected into certain strains of mice induces hypoglycemia, among many other biological effects (1). Most of these effects are known to be associated with substances found only in smooth virulent cultures of *B. pertussis*. Some antigens, however, are lost during the transformation from virulent smooth to nonvirulent rough forms (2). *B. pertussis* vaccine is also known to cause hypoglycemia in experimental animals (3-5). It has further been shown in preliminary clinical trials that the vaccine has a temporary therapeutic effect in the treatment of insulin resistant diabetes in human subjects (6). The hypoglycemia causing factor is reported to be associated with the HSF (Histamine Sensitizing Factor) and other factors in

the preparation "pertussigen" by Munoz *et al.* (2).

No method, however, is available in the literature for the purification of the hypoglycemic factor *per se*, from *Bordetella pertussis* cells. The main problem in purification appears to be the extraction of these factors in an aqueous soluble form (7).

In the present studies, hence, three methods of extraction which are reported to yield aqueous soluble preparations from *B. pertussis* cells have been undertaken, and their hypoglycemic effects compared in Haffkine strain mice. This is essential, prior to choosing the suitable extraction method for further detailed studies on the hypoglycemic factor.

Materials and Methods :

Bordetella pertussis strain 25525, a

standard vaccine strain supplied by the H.B.P.C.L. (Haffkine Bio-Pharmaceutical Corpn. Ltd.) was used in the present studies. The cells were cultured in the laboratory on standard Bordet-Gengou blood agar slants and cultivated in Verwey's liquid media in the usual manner (8). Further, proper procedural care was exercised during cultivation of cells to ensure that purity is maintained and there is no phase variation (9). The yield of cells finally obtained was 90 to 100 billion cells/ml, the cell counts being determined by the standard WHO International Opacity Reference for *Bordetella pertussis* (10). The cells were centrifuged, washed with saline and preserved as a paste at -15°C or acetone dried. Alternatively, the cells were killed by formalin treatment (0.5%) prior to centrifugation.

The alkaline saline extract (SE) was prepared according to Munoz and Hestekin (11). The cells were disrupted mechanically at $4-5^{\circ}\text{C}$, saline was added, pH adjusted to 8.5 with 1N NaOH and centrifuged. The supernatant was decanted, pH adjusted to 7.4 with 0.1N HCl, dialyzed and then lyophilized.

For extraction using Triton X-100 (7), the cells were suspended in extraction buffer, pH 8, containing 1M sodium chloride, 0.05M sodium pyrophosphate and 0.5% Triton X-100 (Triton buffer). The suspension was centrifuged at 27,000 g and the clear soluble supernatant (TE) saved for the study.

In the third extraction procedure (2) employing enzymes, the cells were suspended in Tris-buffer pH 7.5, containing 100 mg lysozyme, 2.5 mg

DNase and 2.5 mg RNase. The final precipitate obtained after ammonium sulphate precipitation, was dissolved in 1M NaCl and 0.05 M sodium pyrophosphate buffer, pH 7.4. The extract (EE) was dialyzed against distilled water and then used in the studies.

The above extracts were concentrated by vacuum dialysis and protein concentration was measured by the method of Lowry *et al.* (12). Blood sugar was assayed by administering the preparations to Haffkine Strain mice (female, 18-20 gms). Three groups of mice (ten in each) were given the extracts adjusted to identical protein concentration, in 0.5 ml, intraperitoneally (i.p.). The control group (ten mice) received 0.5 ml saline. Fasting blood glucose levels (18 hours) were determined by the glucose oxidase method (13).

Results and Discussion :

It is reported that the HSF with which the hypoglycemic factor is associated, is more soluble at pH higher than 7 and this activity can be completely precipitated out of solutions at pH 4.8 (11). These points have been taken into consideration in the above extraction methods. The hypoglycemic effects of the three extracts are given in Table 1.

It is seen that as compared to control value, the three extracts show significant lowering of fasting blood glucose levels in mice, the percentage lowering being 13 to 25%. No significant difference, however, is observed between the hypoglycemic effects of the three individual extracts; the values being 54.4 ± 6.07 ; 57.85 ± 7.5 and

63.1 ± 6.69 for SE, TE and EE, respectively.

The preparation "Pertussigen" with which the hypoglycemic effect is associated, is relatively rich in the outer membrane proteins present in Phase I strains of *B. pertussis* (14). The above three extraction methods are attempts to solubilize the membrane proteins and obtain aqueous extracts.

The saline extracts (SE) contain various antigens and according to Munoz *et al.* (1), none of the several electrophoretic and chromatographic procedures attempted, were completely successful in obtaining contaminant free preparation of "Pertussigen". Further, the yields were also low. In another study (15) it was found that $MgSO_4$ precipitation and chromatography of SE yielded only partially purified fractions, apart from their relatively high nucleic acid content.

Triton X-100 extraction though leads to complete solubilization of membrane proteins, is reported to cause problems in gel electrophoresis (16). The enzyme preparation which contains lysozyme, on the other hand, leads to selective solubilization of outer membrane proteins (17). In addition, the onestage precipitation step involved in the preparation of the extract (EE), makes it free from many contaminants. However, it appears that some loss in activity is unavoidable particularly after successive steps of purification of the lysate (2).

Further purification, and characterization of the hypoglycemic factor from the enzyme extract (EE) is underway.

Acknowledgement :

The award of a research scheme by the Indian Council of Medical Research is gratefully acknowledged. We thank Dr. A. J. Baxi for his helpful suggestions. The authors wish to thank the Director, Haffkine Institute for providing facilities to carry out the present work.

Table 1

Comparative blood glucose values of Bordetella pertussis extracts prepared by 3 different methods. Protein concentration was arbitrarily adjusted to 600 $\mu g/ml$.

Experiment	Control (0.5 ml)	Alkaline saline extract (SE) (0.5 ml)	Triton X-100 extract (TE) (0.5 ml)	Enzyme treated extract (EE) (0.5 ml)
Route	i.p.	i.p.	i.p.	i.p.
Protein concentration	—	300 $\mu g/m$	300 $\mu g/m$	300 $\mu g/m$
Blood glucose (mg %)	72.8 ± 3.96 SD	54.4 ± 6.07 SD*	57.85 ± 7.5 SD*	63.1 ± 6.69 SD*

*P < 0.01; N = 10

REFERENCES

1. Munoz, J. J., Bergman, R. K., Cole, R. L. and Ayers, J. C. (1978) — Purification and activities of Pertussigen, a substance from *Bordetella pertussis*. *Toxins: Animal, Plant and Microbial.*, P. Rosenberg (Ed.), Pergamon Press, New York, 1015-29.
2. Munoz, J. J. and Bergman, R. K. (1979) — Mechanism of action of Pertussigen, a substance from *Bordetella pertussis*. *Microbiology*, American Society for Microbiology, Washington, D. C., 193-7.
3. Perfentjev, I. A. and Schleyer, W. L. (1949) — The influence of Histamine on the blood sugar level of normal and sensitized mice. *Arch. Biochem.*, **20**, 341-6.
4. Stronk, M. G. and Pittman, M. (1955) — The influence of Pertussis vaccine on Histamine sensitivity of rabbits and guinea pigs and on the blood sugar in rabbits and mice. *J. Infect. Dis.*, **96**, 153.
5. Dhar, H. L. and West, G. B. (1972) — Sensitization procedures and blood sugar concentration. *J. Pharm. Pharmac.*, **24**, 249-50.
6. Dhar, H. L., Dhirwani, M. K. and Sheth, U. K. (1975) — Pertussis vaccine in diabetic requiring high dosage insulin, *Brit. J. Clin. Pract.*, **29**, 119-20.
7. Munoz, J. J. and Cole, R. L. (1979) — Extraction of Pertussigen from *Bordetella pertussis* with aid of Triton X-100. *IRCS Medical Science*, **1**, 218.
8. Verwey, W. F., Thiele, E. H. Sage, D. N. and Schuchardt, L. F. (1949) — A simplified liquid culture medium for the growth of *Hemophilus pertussis*. *J. Bact.*, **58**, 127-34.
9. Leslie, P. H. and Gardner, A. D. (1931) — The phases of *Haemophilus pertussis*. *J. Hygiene*, **31**, 423-34.
10. Spasojevic, B. (1979) — Virulence of *Bordetella pertussis* strains for mice by the intracerebral route and the comparison of virulence and toxicity. *Microbiology*, **16**, 67-74.
11. Munoz, J. J. and Hestekin, B. M. (1962) — Method of preparing Histamine sensitizing factor. *Nature*, **196**, 1192-93.
12. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) — Protein measurement with Folin-Phenol reagent. *J. Biol. Chem.*, **193**, 267-75.
13. Huggett, A. and Nixon, D. A. (1957) — Use of glucose oxidase, peroxidase and o-dianisidine in determination of blood and urinary glucose. *Lancet*, **2**, 368-70.
14. Dobrogosz, W. L., Ezzell, J. W., Kloos, W. E. and Manclark, C. R. (1978) — Physiology of *Bordetella pertussis*, International Symposium on Pertussis, Ed., Manclark, C. R. and Hill, J. C., U. S. Department of Health, Education and Welfare, NIH., 86-93.
15. Ramakrishnan, P. (1979) — Studies on the hypoglycemic effect of fractions from MgSO₄ precipitate of *Bordetella pertussis* extracts. *Bulletin of Haffkine Institute*, **7**, 1-6.
16. Nakashima, H., Nakagawa, Y., Makino, S. (1981) — Detection of the associated state of membrane proteins by poly-acrylamide gradient gel electrophoresis with non-denaturing detergents. *Biochimica et Biophysica Acta*, **643**, 509-18.
17. Davis, B. D., Dulbecco, R., Eisen, H. N., Ginsberg, H. S. and Wood, W. B. (1973) — *Microbiology*, 2nd Ed., Harper and Row, London, 107-27.

Food Habits of the Lesser Bandicoot Rat *Bandicota bengalensis*

D. M. RENAPURKAR and P. J. DEORAS

Department of Zoonosis, Haffkine Institute, Parel, Bombay 400 012, India.

A study of food habits of the lesser bandicoot rat *Bandicota bengalensis* was undertaken by excavating *B. bengalensis* burrows in wheat, jowar and paddy growing areas. It was observed that the food stored in the burrows was mainly obtained from the food grown in the locality. It is, therefore, felt that the food habits of the rat *B. bengalensis* depended on the availability of local food.

Introduction

Bandicota bengalensis, the lesser bandicoot rat, is widely distributed all over India. This rat constitutes to be the principal commensal rodent species in Greater Bombay area and is a major field rat producing damage to food crops throughout the Maharashtra State. Despite this, very few studies have been carried out to record its food habits and data available on its ecology and food habits are meagre (Barnett, 1951 ; Deoras, 1964 ; Chitre and Deoras, 1968). In order to obtain information on food preferences, a study was undertaken to record the different types of food material stored by these rodents in their burrows.

Materials and Methods

Three distinct areas where wheat, jowar and paddy crops are individually grown, were selected. Burrows situated in the fields and their neighbourhood (within a distance of 1 km) were dug out and burrow contents were collected separately and transported to laboratory or a detailed study. Wherever possible, rats were trapped by using multiple rat

catch traps called wonder traps. The trapped rats were killed and their stomach contents were analysed. The study areas were situated in Kolaba, Nasik and Beed districts of Maharashtra State.

Observations and Results

Table 1 shows details of food contents recovered from burrows in different areas. It will be seen from the Table that in jowar growing area the food collected in burrows primarily consisted of jowar (*Sorghum vulgare*). In wheat growing areas it principally consists of wheat (*Triticum aestivum*) and in paddy area it is paddy (*Oryza sativa*). The food was stored as ear heads, grains, dry leaves.

Examination of burrows situated in grain shops in Panvel area near Bombay showed that these burrows stored a variety of material including cereals, groundnut, potatoes, vegetables, pulses, etc. The material recovered from 90% burrows was in intact condition. An analysis of stomach contents of 100 rats

Table 1
Food material observed in the burrows of *Bandicota bengalensis*.

Place	Major crops	No. of burrows observed	Percentage of burrows showing food material				Remarks
			Paddy	Jowar	Wheat	Other items	
Panvel	Paddy fields	125	100	—	—	—	
Niphad	Wheat fields	100	—	—	100	—	
Niphad	Jowar fields	25	—	100	—	—	
Gevrai	Jowar fields	100	—	100	—	—	
Panvel	Shops	25	30	20	20	20*	Pulses, ground-nut, potatoes, vegetables, etc.
Sewri	Fish godown	12	—	—	—	100	Dry fish

trapped from fish godowns and examination of their burrows showed that these rats thrived principally on dried fish as revealed by their stomach contents and stored dried fish material observed in their burrows.

Discussion

Several workers have shown that food habits of rodents mainly depend on the environmental factors and availability of particular type of food in a locality (Barnett, 1960 ; Deoras, 1968 ; Majumdar *et al.*, 1966 ; Spillet, 1966 ; Renapurkar, 1971). Our observations also

reveal a similar phenomenon in the field rat *Bandicota bengalensis*. This rat is found to feed on jowar, wheat, rice, dry fish, vegetables and store these items in their burrows according to their availability. It will be interesting to study its food pattern in areas where a variety of food is available and multiple cropping is undertaken. This will enable us to understand its preferences and help us to determine the extent of damage done to various crops by this pest. A study on these lines in the future will certainly prove very useful.

REFERENCES

1. Barnett, S. A. and Spencer, M. M. (1951). Feeding, social behaviour and interspecific competition in wild rats. **Behaviour**, **3**, 229-242.
2. Barnett, S. A. (1966). The feeding of rodents. **Proc. Ind. Rod. Symp. Calcutta**, **113-123**.
3. Chitre, G. D. and Deoras, P. J. (1966). Observations on the feeding propensity and behaviour of rats. **Proc. Ind. Rod. Symp. Calcutta**, **145**.
4. Deoras, P. J. (1964). Annual Reports of the coordinated scheme for study of field rats. I.C.A.R. Reports, 1960-64, ICAR, Delhi.
5. Deoras, P. J. (1968). Cereal intake, preference, pellet exudation by *Rattus rattus* and their significance, **Zeitschrift Agnew Zool.**, **55**, 447.
6. Majumdar, S. K. Krishnakumari, M. K. and Yashoda, L. V. (1966). Some observations on food preferences and intake of rats under different ecological conditions. **Proc. Ind. Rod. Symp. Calcutta**, **124-136**.
7. Renapurkar, D. M. (1971). Some plague epidemiological studies in Maharashtra State, 248-251.
8. Spillet, J. J. (1966). Population studies of the lesser bandicoot rat in **Calcutta Proc. Ind. Rodent Symp.**, **Calcutta**, **84-92**.

Binding of Monoene Bhilawanol on *Clostridium tetani*

*B. K. PATWARDHAN, U. R. PHADKE and R. B. GHOOI

Haffkine Institute, Parel, Bombay 400 012.

Monoene and diene bhilawanols inhibit the growth of *Clostridium tetani* *in vitro*. In an attempt to localize the site of action of the monoene, the binding of the compound to different subcellular fractions has been studied, using high pressure liquid chromatography to quantitate the compound. The study reveals that a major amount of the compound is bound to the cell wall fraction.

Introduction :

Bhilawanol, a major constituent of *Semecarpus anacardium* occurs naturally with one or two double bonds in its side chain. The monoene and the diene have been subjected to detailed chemical¹ and biological studies.² In the studies on aerobes and anaerobes it has been demonstrated that these compounds possess specific inhibitory activity against *Cl. tetani*, but were without effect on aerobes.³

The low minimum inhibitory concentrations (2.5 and 1.25 μ g/ml, for the monoene and diene respectively), seemed to point to potent inhibitory effects of the compounds. These studies necessitated further work to pin point the mechanism of action, as a part of which localization of the binding site was tackled to start with.

The monoene and the diene have been described only recently, and there was a dearth of methods for estimation of the compounds. For this reason a method was developed, using high

pressure liquid chromatography, for distinguishing between and quantifying these compounds.

Material and Methods :

Bhilawanol monoenes and dienes were supplied by Dr. U. R. Nayak of the National Chemical Laboratory, Pune. The monoene was checked for its purity on HPLC and found to have contamination of diene and an unidentified component totally accounting to about 20% w/w.

A 24 hours old culture of *Cl. tetani* (Harvard strain, H.B.P.C.L., Bombay) was subcultured in two flasks (100 ml each) and incubated for 24 hours at 35° C. The cells were centrifuged and washed in saline and resuspended in saline containing 0.5% thioglycollate. Keeping one flask as control, to the other was added 20 mg of bhilawanol monoene, and incubation continued for another 30 minutes. The cells were again centrifuged, washed and resuspended in saline.

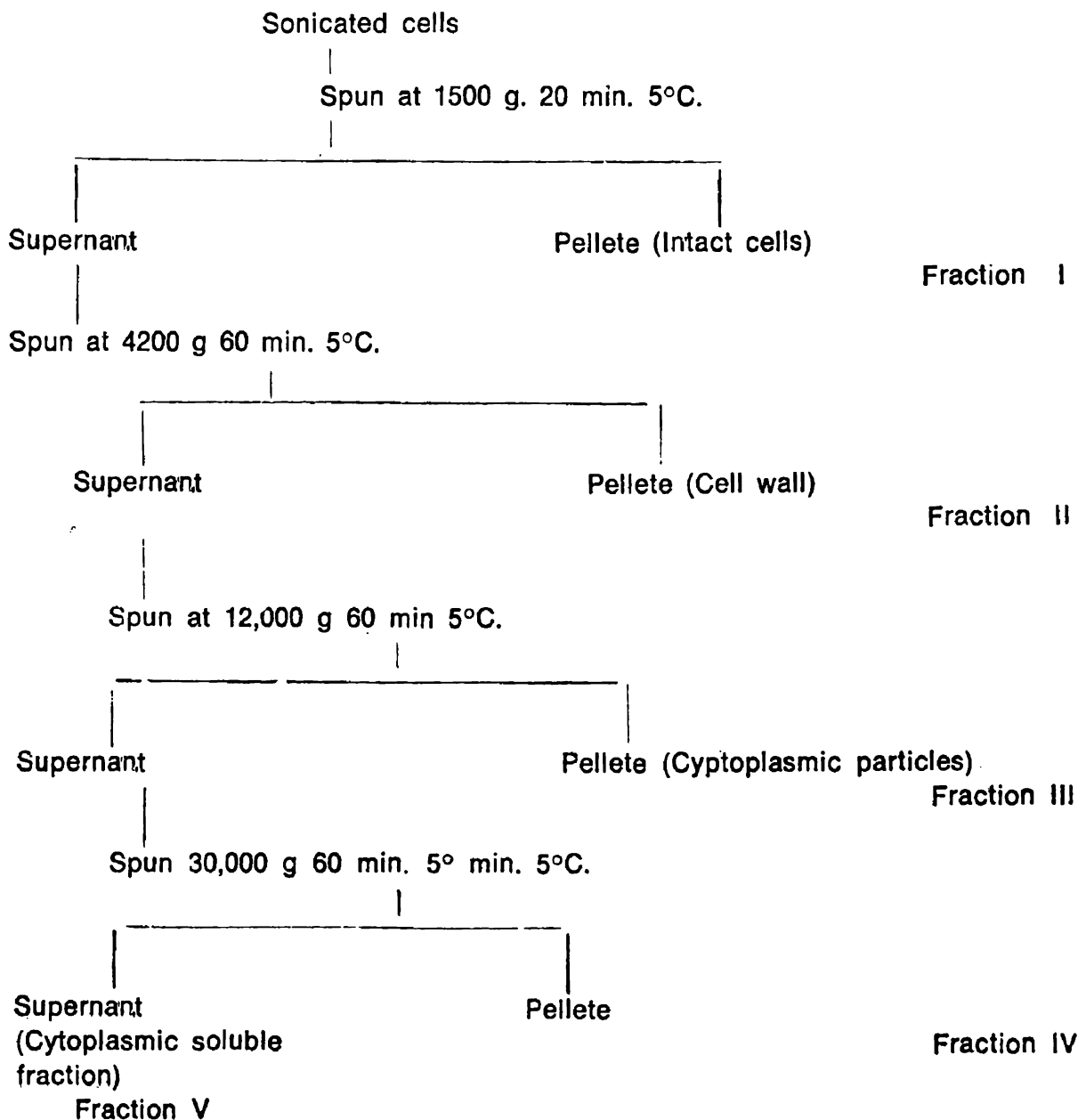
* Present Address : Department of Botany and Microbiology, University of Pune - 411 007.
Received for publication : 1-9-82.

The cells were killed by ^{60}Co irradiation in Gamma cell 100, Atomic Energy Canada Ltd., with a dose of 30,000 Rads. The killed cells were disrupted in Sonifier B-30 sonicator for 15 minutes at 20 K.Hz. (50% duty cycle). The disrupted cells were separated into different sub-

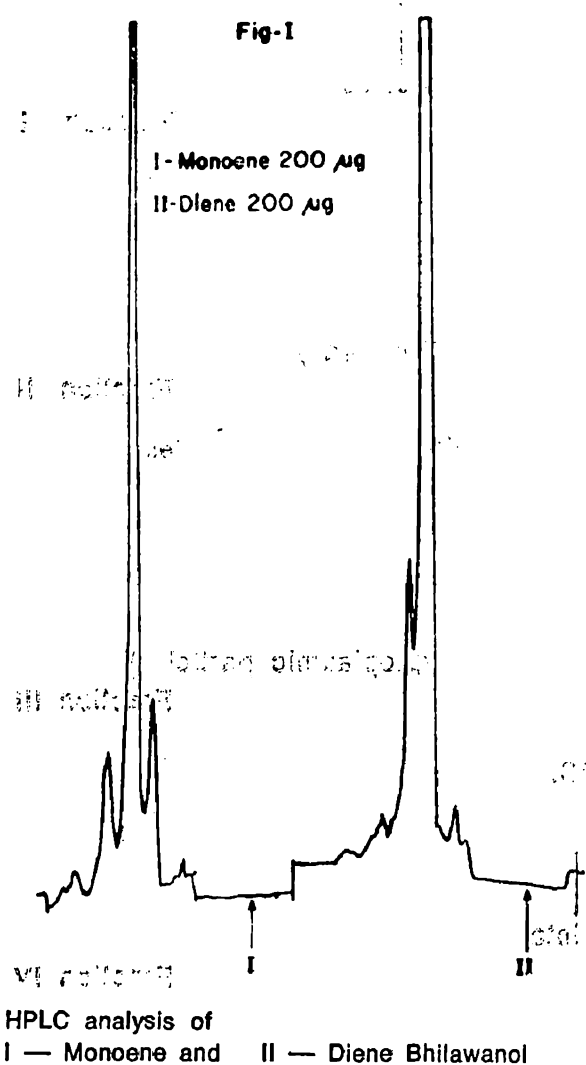
cellular components⁴ as shown in scheme 1, using a Beckman J 21 C refrigerated centrifuge. The different fractions were repeatedly extracted with chloroform G.R., and the extracts dried *in vacuo* and redissolved in 100 μl of methanol.

Scheme 1

Separation of Subcellular Components of *Cl. tetani*



The monoene was quantitated on an Altex high pressure liquid chromatograph using 254 U. V. detector. The column used was an Ultrasphere Octyl reverse phase column. Solvent for elution was Methanol with a flow rate of 1 ml/minute, the monoene being detected in an 8 μ l optical cell and 0.16 AUF sensitivity. Using standard monoene the retention time and the amount of monoene per extract was calculated. High pressure liquid chromatography techniques⁵ were preceded by TLC of the extracted monoene using chloroform solvent on silica gel plates.



Results :

Preliminary TLC of extracted monoene suggested the presence of a high amount of the compound in the cell wall of the cells. High pressure liquid chromatography studies demonstrated that the monoene was contaminated with diene and an unknown compound to the extent of about 20% w/w, the retention time of monoene and diene being 270 and 252 seconds respectively.

A standard curve for UV absorption versus amount of monoene at 0.16 AUF, was plotted for amounts ranging from 1.5×10^{-5} to 2.4×10^{-4} mM. The extracts of subcellular fractions were found to contain the monoene in amounts shown in table 1. From this table, it was found that monoene was present in highest concentration in the cell wall fraction of the cell.

Table 1

Monoene Concentration in Subcellular fractions of *Clostridium tetani*

Fraction No.	Peak height in units	Concentration in mM Monoene
I	4.5	9.95×10^{-5}
II	13.5	2.2875×10^{-4}
III	4.0	1.5×10^{-5}
IV	Not detected	—
V	"	—

Discussion :

One of the fundamental principles of pharmacology is that a drug should fix to some subcellular organelle prior to producing an effect. The site of action

becomes all the more important where an agent inhibits the growth of microbes, in view of the likely toxicity of the agent on the host.

A number of antimicrobial agents have been so far reported which interfere with bacterial growth without entering the bacteria. The present data indicates that the monoene penetrates the cell to no appreciable extent. The concentration of the monoene in cell wall was found to be 20 to 30 times higher than in other fractions, suggesting that the monoene binds to the cell wall and in actuality this binding may lead to a growth inhibition.

The present studies are admittedly too preliminary to draw any conclu-

sion about the mechanism of action of the monoene ; yet, in view of its strong binding to the cell wall components studies on the effects of the monoene on the cell wall of *Cl. tetani* are indicated.

Acknowledgements :

The authors are grateful to The Director, Haffkine Institute, for the facilities provided and to The Science and Technology Cell, Govt. of Maharashtra, and The Department of Science and Technology, Govt. of India for financial support. The Director of C.C.R.A.S. is also gratefully thanked for financial support to U.R.P. Grateful thanks to Dr. U. R. Nayak National Chemical Laboratory, Pune for his kind help.

REFERENCES

1. T. R. Govindachari, B. S. Joshi, V. N. Kamal and N. Vishwanathan (1971) — *Ind. J. Chem.* **9**; 1044 (1971).
2. R. B. Ghooi, B. K. Patwardhan, K. D. Sharma (1982). — A paper presented at VIIIth Southern Regional Pharmacological Conference held in May 1982, Bangalore.
3. B. K. Patwardhan, R. P. Francis, S. V. Kapre, and K. D. Sharma. (1982) — *Bull. of Haff. Inst.* **10** : 27-30 (1982).
4. Kazuo Ryoyama, Shunnojoke Natsuume-saki, Harumi Hirota and Saburo Koshimura (1981) — *Japan J. Expt. Med.* Vol. 51, p. 335 — 344 (1981).
5. A. S. Gupta and Sukh Dev. (1983) — *J. Chromat.* **12**:189 (1963).

Individual and Synergistic Activity of some Essential Oils as Mosquito Larvicidal Agents

S. R. CHAVAN, NEENA P. SHAH and S. T. NIKAM

Department of Phytochemistry,
Haffkine Institute, Parel, Bombay - 400 012.

In view of the pressing need for vector control agents from indigenous plants, the essential oil from the following plants individually and in combination were studied for mosquito larvicidal activity: *Ocimum sanctum* Linn, *Ocimum basilicum* Linn and *Eucalyptus globulus* Labill.

Ocimum sanctum Linn essential oil showed 16% mortality with 0.01% concentration. In the case of *Eucalyptus globulus* Labill oil 16% mortality was observed with 0.002% concentration, whereas *Ocimum basilicum* Linn essential oil resulted in 8% mortality at the concentration of 0.002%.

Combination of the above three essential oils produced 100% mosquito larvicidal (*Culex P. fatigans*) activity.

Introduction :

Production and use of essential oils did not become common until the second half of the sixteenth century. The main progress regarding the nature of the essential oils and methods of their preparation was published in 1561 by Canrad Gesier, in his book "The Saurus Euonymi Philiatr". Due to revolution in the science of chemistry during 18th century there was a new and illuminating approach to the investigation of the nature of essential oils. In 19th century, gradually the use of essential oils in the medicinal drugs became quite subordinate to their employment in the production of perfumes, beverages, foodstuffs, etc. This led to the investigations of essential

oils and elucidation of their compounds for the use in solving various day to day problems of life. One such problem has been to control those insects which have behaved as serious foes towards mankind. Thus, the examination of essential oils for insect control properties is a sound proposition.

The problem is of course difficult due to the relative differences in the susceptibility of the various life stages of insects to such chemical treatments. Nevertheless, various essential oils or compounds isolated from them have been used in the form of insect attractants, repellent and insecticides. In our investigation, of essential oils from *Ocimum sanctum*, *Ocimum basilicum* and *Eucalyptus globulus* as mosquito

larvicidal agents, we have studied their activity individually and in combination.

Yield of *Ocimum sanctum* was found to be 0.7%, containing 71.3% eugenol, 3.2% carvacol, 20.4% methyl eugenol and 0.7% cariophylline. This oil is used as insect repellent (1). Fresh flowering herbs of *Ocimum basilicum* yield essential oil containing linalool 65.3%, small amount of cineole, eugenol sesquiterpene and d-terpene (2). Mature plants yield 0.4% essential oil (3); leaves yield 0.5% essential oil containing methyl cinnamate 56.67%, linalool 4.36% and terpinine (4). The essential oil from the plant during rainy season contains 69.66% methyl cinnamate and 20.53% linalool (5). Leaves of *Eucalyptus* yield 0.92 to 1.2% essential oil containing cineole 62%, pinene 24%, sesquiterpene, alcohols (6). Decoction of leaves is used as insect and varmine repellent.

Essential oil from *Eucalyptus* has shown ovicidal effect. Carvacol, eugenol, pinene and cineole were reported to possess larvicidal/insecticidal activity against *Aedes aegypti* and *Musca domestica*.

It has already been reported by Chavan *et al.*(7) that petroleum ether extract of *Ocimum sanctum* Linn possesses good mosquito larvicidal activity. The larvicidal activity of essential oil from *Ocimum basilicum* and its synergistic effect in combination with Finit was studied against IV stage larvae of *Culex P. fatigans* (8).

Materials and Methods :

Leaves of *Ocimum sanctum* Linn, *ocimum basilicum* Lim and *Eucalyptus*

globulus Labill were cut into pieces and subjected to steam distillation. The distillate was then extracted with petroleum ether (40-60°) and petroleum ether extract was dried on anhydrous sodium sulphate. Petroleum ether was removed carefully on controlled temperature water-bath. The essential oils from these three plants were recovered separately, the yield of the essential oil from *O. sanctum*, *O. basilicum* and *E. globulus* was found 0.6%, 0.5% and 0.8% respectively. Toxicity of these essential oils to mosquito larvae (*Culex p. fatigans*) IV stage was evaluated separately and in combination as per W.H.O. specifications (9).

Twenty five IV instar larvae were taken in 500 ml beaker containing 250 ml tap water. The essential oils were used in different concentrations. Solvent (2 ml acetone) control and water control was taken simultaneously. Each experiment was carried out in triplicate.

Results and Discussion :

Table 1 shows mosquito larvicidal activity of essential oils from *O. sanctum* (Tulsa), *O. basilicum* (Subja) and *E. globulus* (Nilgiri) against *Culex p. fatigans*. It can be seen that Tulsa at low concentration (0.002%) produced 8% mortality, whereas 100% mortality was obtained at 0.02% concentration. With Nilgiri essential oil low concentration (0.0004%) possessed 8% mosquito larvicidal activity and 0.01% produced 100% mortality. Similarly Subja oil exhibits 8% mortality of mosquito larvae at 0.002% concentration and 100% mortality with 0.012% concentration.

Table II shows synergistic effect of these three essential oils. It can be clearly seen that *O. sanctum* (Tulsa), *O. basilicum* (Subja) and *E. globulus* (Nilgiri) essential oils in the concentrations of 0.01%, 0.002% and 0.002% showed 16%, 8% and 16% mortality; whereas, combinations of these three concentrations produced 100% mortality.

There are very few reports available regarding essential oils being synergistic in nature. But in our results (Table II) significant synergistic effect is observed by the combination of essential oils obtained from *O. sanctum* Linn, *O. basilicum* Linn and *E. globulus* Labill. This gives the important clue that these oils separately can be tried for their synergistic effect with known insecticides. Besides this, *E. globulus* being easily available can give a chea-

per and safer insecticide with probable synergistic effect. In fact, a valuable source of chemicals which may be developed as new vector control agents after further studies on *E. globulus* chemical constituents and their evaluation as a synergistic agent in combination with known insecticides.

Acknowledgements :

The authors are thankful to Dr. K. D. Sharma, Director, Haffkine Institute, Bombay for constant encouragement in this work. They are also grateful to Dr. D. M. Renapurkar, Asstt. Director, Zoonosis Department for supplying mosquito larvae (*Culex p. fatigans*). Thanks are also due to Mr. H. V. Nerurkar for collection of plant material, Mr. V. R. Patade and Shri D. T. Jadhav for laboratory assistance.

Table 1

Mosquito Larvicidal Activity of Essential Oil from *Ocimum Sanctum* Linn, *O. Basilicum* Linn and *E. Globulus* Labill.

Essential oil from	% Concentration (ml/ml)	% Mortality (24 hrs)
1. <i>Ocimum sanctum</i> Linn (Tulsa)	0.002	8
	0.01	16
	0.02	100
2. <i>Ocimum basilicum</i> Linn. (Subja)	0.002	8
	0.004	40
	0.008	80
	0.012	100
3. <i>Eucalyptus globulus</i> Labill. (Nilgiri)	0.0004	8
	0.002	16
	0.004	40
	0.008	84
	0.01	100

Table 2
Synergistic Effect of Essential Oils from *O. Sanctum* Linn, *O. Basilicum* Linn, and *E. Globulus* Labill.

Essential oil from	% Concentration (ml/ml)	% Mortality (24 hrs)
1. <i>O. Sanctum</i> Linn	0.01	16
2. <i>O. basilicum</i> Linn	0.002	8
3. <i>E. globulus</i> Labill	0.002	16
4. Combination of above three oils with same (above) concentrations.		100

REFERENCES

- 1 Dutt, S. (1940) — New Indian essential oils. **Chem. Abstr.**, 6015.
- 2 Nelson, E. K. and Lowman, M. S. (1936) — Chemical composition of sweet basil oil. **Chem. Abstr.**, 1941.
- 3 Chopra, R. N., Nayar, S. L. and Chopra, I.C. (1956) — Glossary of Indian Medicinal Plants, C.S.I.R., New Delhi, p. 179.
- 4 Nigam, S. S. and Dutt, S. (1948) — Chemical examination of essential oil derived from *Ocimum basilicum* (album). **Chem. Abstr.**, 4717.
- 5 Khorana, M. L. and Wangikar, M. B. (1950)— *Ocimum basilicum* chemical study of oil. **Indian J. Pharm.**, 12 (5), 132-135.
- 6 Wealth of India, Vol. III, p. 207.
- 7 Chavan, S. R., Deshmukh, P. B. and Renapurkar, D. M. (1979) — Investigation of indigenous plants for larvicidal activity. **Bull. Haff. Instt.**, 7, 23-33.
- 8 Chavan, S. R. and Nikam, S.T. (1982) — Mosquito larvicidal activity of *Ocimum basilicum* Linn. **Indian J. Med. Res.**, 75, 220-22.
- 9 W.H.O. (1960) — Insecticidal resistance and vector control. *Tenth Report of World Health Organisation Expert Committee on Insecticides*, 191, 98.

DIRECTIONS TO CONTRIBUTORS

Bulletin of Haffkine Institute is a triannual publication. It embodies review and research articles in biomedical sciences, mainly devoted to studies on Communicable Diseases.

Contributions should be precise and concise, highlighting original observations and inferences. The type-script (T.S.) should be sent in duplicate, typed in double space on only one side of the sheet with ample margins. A brief summary not exceeding 200 words, should accompany the article. The title should be informative and specific, followed by author's name and address.

A short title in not more than forty letters should also be suggested. The text of the paper should be generally sub-divided into: Introduction, Materials and Methods, or Experimental, Results, Discussion, Acknowledgement and References.

'Short Abstract' of about 100 words suitable for inclusion in 'International Abstracts of Biological Sciences', etc. should be typed on a separate sheet, with author's name, address and title of the paper.

Results should be presented concisely in tables giving only important and relevant data and avoiding incorporation of elaborate observations.

Restraint should be exercised in submitting illustrative matter. Photographs should be well-defined and on glossy paper.

References in the text should be cited by numbers serially as they appear, and to be inserted in parenthesis, e.g., Plasmid-mediated resistances to these heavy metals have also been observed in enteric bacilli, especially in P factor carrying organisms (1), and have recently been studied by several workers (2, 3, 4). References at the end should be arranged numberwise serially as they figure in the text with author's name first and abbreviations according to Index Medicus. The following pattern may be followed:

- (i) Reuben, R.; Carey, D. E. and Meyers, R. M. (1968) — Japanese encephalitis studies in Vellore, S. India, *Indian J. Med. Res*; **56**, 109-18.
- (ii) Cruickshank, R. (1968) — *Medical Microbiology*, 11th Ed. 180-4, E and S Livingstone Ltd., Edinburgh, p. 28.

Twenty-five reprints will be supplied to the author/s free of cost. Additional reprints may be purchased and should be ordered when galley-proof is returned.

All correspondence should be addressed to the Editor, Bulletin of Haffkine Institute, Haffkine Institute for Training, Research and Testing, Parel, Bombay 400 012 (India).

Annual Subscription : Rs. 20 (Inland) Rs. 25 (Foreign)

Edited and published by Dr. V. R. Deshpande, Director, Haffkine Institute for Training Research and Testing, Bombay 400 012 and printed at Vijay Mudran, Bombay 400 031.

