Evaluation of Biolarvicides

Biolarvicides

Over the last few decades, there has been growing realisation that alternative methods to chemical control of vector mosquitoes should be developed. One such method is the use of *Bacillus sphaericus* and *B. thuringiensis* var *israelensis (Bti)* Commercial formulations of *Bti* are available and can be used in large-scale mosquito control operations. The major advantages of biolarvicides are—reduced application costs, safety to environment, human beings, animals and other non-target organisms. Various formulations of *B. sphaericus* and *B. thuringiensis* var *israelensis* have been evaluated during the last two decades.

Laboratory and Field Evaluations

Biocide-S

Biocide-S (*B. sphaericus* 1593M) developed by Madurai Kamraj University was the first biocide tested at NIMR in 1983, later three experimental formulations of Biocide-S–HIL-8, 9 and 10 wettable powder and dust formulations were developed and tested in laboratory and small-scale field trials (Mittal *et al* 1985). These formulations were found to be effective against both *Culex* and *Anopheles* larvae in laboratory and small pits in field. However, further development of various aqueous formulations, MKU-1, 2 AU, etc. based on Biocide-S were not much effective against *Anopheles* sp, particularly *An. culicifacies*. In addition to Biocide-S, various other formulations of *B. sphaericus* obtained through WHO and various other laboratories in India and abroad were tested both in laboratory and small-scale field trials (Ansari *et al* 1989, 1995). All these formulations were, however, effective only against culicines and some anopheline species, particularly against *An. stephensi* and *An. subpictus* and not against *An. culicifacies*.

Solvay, Spherimos and Vectolex

Solvay, a liquid and Abotts granular formulations of *B. sphaericus* 2362 obtained through WHO, were tested against *An. stephensi*, *An. culicifacies* and *Cx. quinquefasciatus* both under laboratory and field conditions (Ansari *et al* 1989). Later two more formulations of *B. sphaericus* 2362 namely Spherimos and Vectolex 2.5 AS obtained through WHO were tested (Ansari *et al* 1995). It was found that *Cx. quinquefasciatus* was more susceptible to all these formulations followed by *An. stephensi* and *An. culicifacies*. In the field condition, absolute mortality in *Culex* larvae was obtained with Spherimos @ 2 ml/m² for one week in pools and 99% reduction

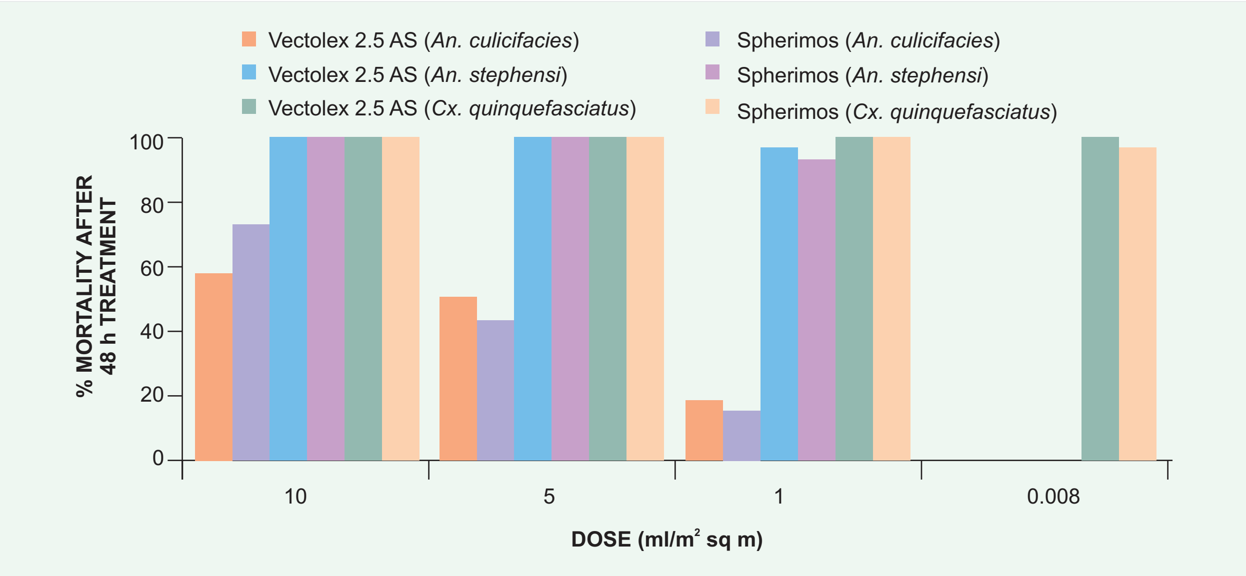

Fig. 64: Laboratory evaluation of *B. sphaericus* 2362 flowable formulations against larvae

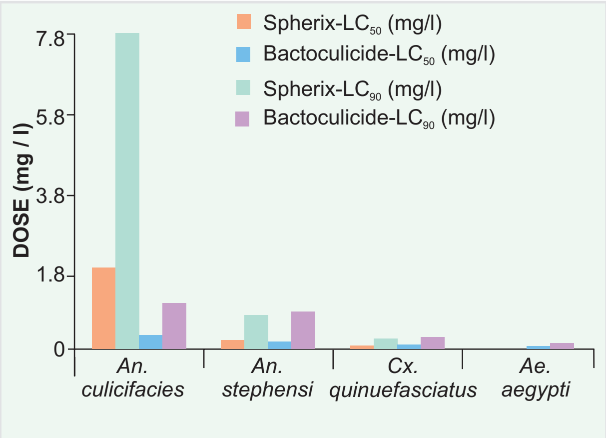

Fig. 65: Comparative toxicities of spherix and bactoculicide against III instar mosquito larvae

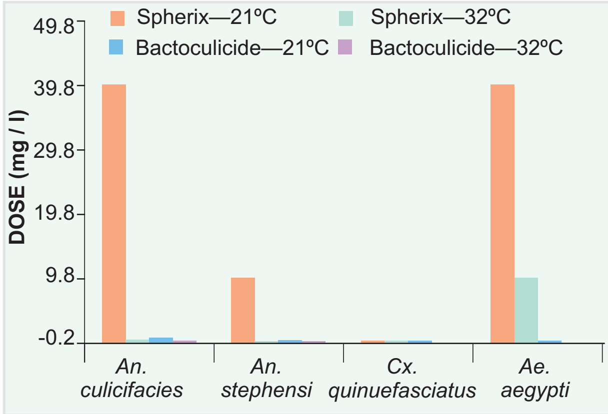

Fig. 66: Effect of temperature on larvicidal activity (LC_{50}) of spherix and bactoculicide against different vector species

for three weeks in the wells. The activity was enhanced for three weeks in pools when the dose was increased to 10 ml/m². On the other hand, against anophelines the absolute impact was seen for one week @ 2 ml/m² and up to 90.8% for two weeks in the pools. In some pools, the impact was moderate. In the wells, the impact was seen for one week @ 2 ml/m², whereas @ 10 ml/m² it was prolonged for two weeks. A good impact of Vectolex was observed on *Culex* sp in the field which lasted for four weeks in the pools @ 2–5 ml/m² and for six weeks in unused wells @ 5–10 ml/m² (Fig. 64).

Spherix or Bactoculicide

In the year 1992, National Malaria Eradication Programme (now NVBDCP) procured two Russian formulations of biolarvicides—Spherix (*B. sphaericus*, serotype H 5a5b, strain B-101) and Bactoculicide (*B. thuringiensis* var *israelensis,* serotype H-14, strain 164). NVBDCP provided 40 MT of Spherix and 10 MT Bactoculicide to NIMR for scientific evaluation by conducting multicentric field trials in different eco-climatic situations in India.

Prior to this, the laboratory bioassays with these two formulations were conducted in Delhi from July to October 1991. It was observed that both Spherix and Bactoculicide were effective against *An. culicifacies*, *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* larvae (LC_{50} values of Spherix ranged from 0.19 to >40 mg/l and Bactoculicide from 0.034 to 0.16 mg/l) (Fig. 65). It was found that Spherix was most effective against *Cx. quinquefasciatus* and the least against *Ae. aegypti,* whereas Bactoculicide was the most effective against *Ae. aegypti* and the least against *An. culicifacies.* Among the two malaria vectors tested, *An. stephensi* was much more susceptible than *An. culicifacies* to both formulations, this observation was also corroborated later by the findings of the field trials (Mittal *et al* 1993).

The safety profile of these formulations to non-target organisms (NTOs) such as *Gambusia affinis*, *Poecilia reticulata*, frog tadpoles, notonectid bugs (*Enithares indica* and *Anisops sardae*) and Cyclops (Copepods) was also established prior to large-scale field trials in the country. The LC_{50} values for above NTOs ranged from 50 to >2000 mg/l, which were many fold higher than the recommended field dose for larval control.

Simultaneously, studies on effect of temperature showed that a 10°C rise in temperature from 21 to 31°C increased efficacy of Spherix and Bactoculicide by 2–100 fold against different vector species (Fig. 66) (Mittal *et al* 1993). This indicated that biolarvicides would lose efficacy in cold climatic conditions and prove very useful in warmer months or tropical climatic conditions which exist in India for most of the year (Fig. 67).

Similarly, it was observed that beyond pH 9.5 the efficacy of both Spherix and Bactoculicide was reduced drastically (Mittal *et al* 1995). There was, however, no significant difference observed in the activity in the pH range 3.5 to 9.5.

Multicentric trials were carried out with Spherix in different NIMR field units, namely at Farrukhabad, Shahjahanpur, Hardwar, Mathura, Ghaziabad, Delhi,

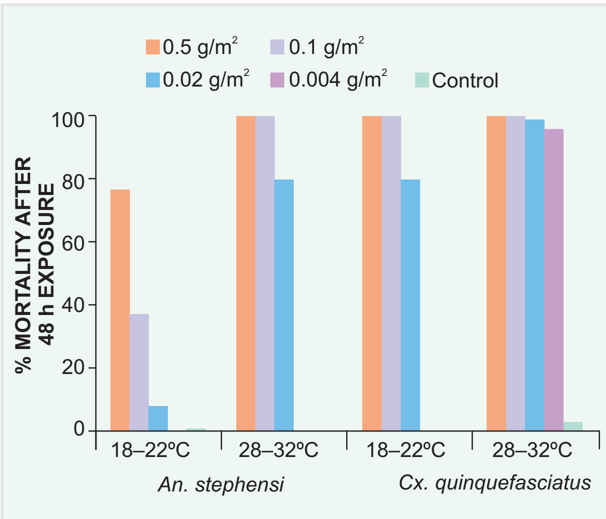

Fig. 67: Studies on the effect of temperature on the efficacy of spherix on *An. stephensi* and *Cx. quinquefasciatus*

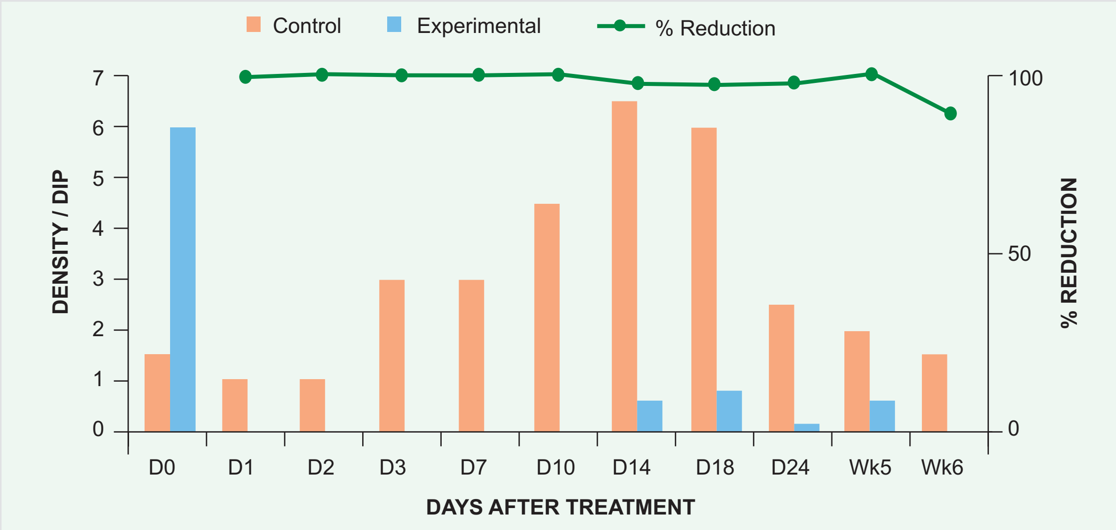

Fig. 68: Efficacy of bactoculicide against mosquito larvae in industrial scraps at BHEL, Hardwar, Uttarakhand

Panaji and Vasco da Gama, Goa, Chennai, Mandla, Nadiad, Shankargarh, Rourkela and Car Nicobar Islands. From the results of these multicentric studies it became evident that both the Russian formulations—Spherix and Bactoculicide were effective against anophelines and culicines including disease vectors in different ecological conditions (Fig. 68). The residual activity of biocides depended upon vegetation, algae and organic pollution. The larval control was longer when breeding habitats were less polluted had less or no vegetation as effect could be seen for up to four weeks in them, whereas the impact lasted for 3–7 days in others (Figs. 69 and 70). Further studies in field and also in laboratory showed the development of resistance to *B. sphaericus*-based biocides but no cross-resistance to *Bti* was observed (Adak *et al* 1995, Mittal *et al* 1998) Later, extensive field trials were also conducted by NIMR to assess the efficacy of the above formulations. Later

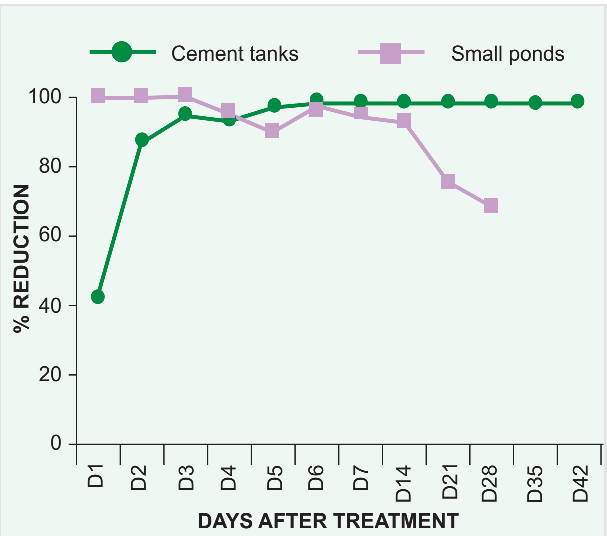

Fig. 69: Larvicidal efficacy of spherix against anopheline larvae in cemented tanks and small ponds after a single application in Bhabhar area, District Nainital, Uttarakhand

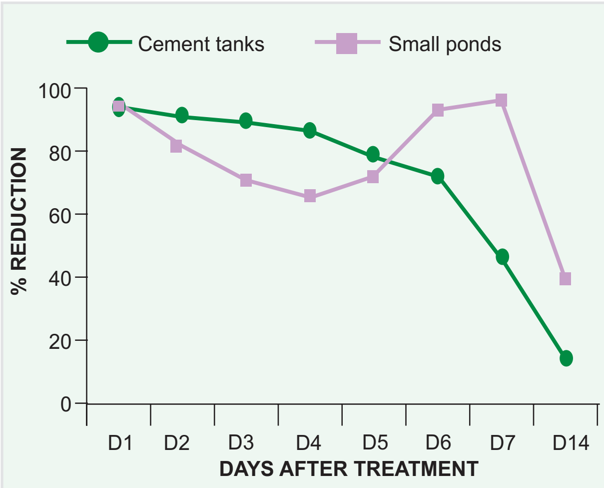

Fig. 70: Larvicidal efficacy of bactoculicide against anopheline larvae in cemented tanks and small ponds

various other formulations of *Bti* tablet, granule and wettable powder against different disease vector species and in urban and rural areas were tested (Batra *et al* 2000; Dua *et al* 1993; Kar *et al* 1997; Kumar *et al* 1994, 1995, 1996, 1998; Mittal *et al* 2000; Shukla *et al* 1997, Yadav *et al* 1997). A commercial formulation (Wockhardt *Bti*) of *B. thuringiensis* var *israelensis* H-14 50% WP was evaluated in field at three NIMR field units at Goa, Haldwani (Uttarakhand) and Shahjahanpur (Uttar Pradesh). Field efficacy was tested in different breeding habitats, namely paddy-fields, river-bed pools, pokhars, pits and drains, septic tanks, ornamental fountains and cement tanks. The formulation was tested at 2 doses, 0.5 and 1 g/m^2. Over 90% reduction in larval density was observed in most of the breeding habitats at a dose of 0.5 g/m^2 except in septic tanks. In septic tanks 1 g/m^2, was effective. Residual effect lasted for one week in most of the breeding sites except in ornamental fountains and cement tanks. In paddy-fields, the efficacy was observed for only two days (Fig. 71).

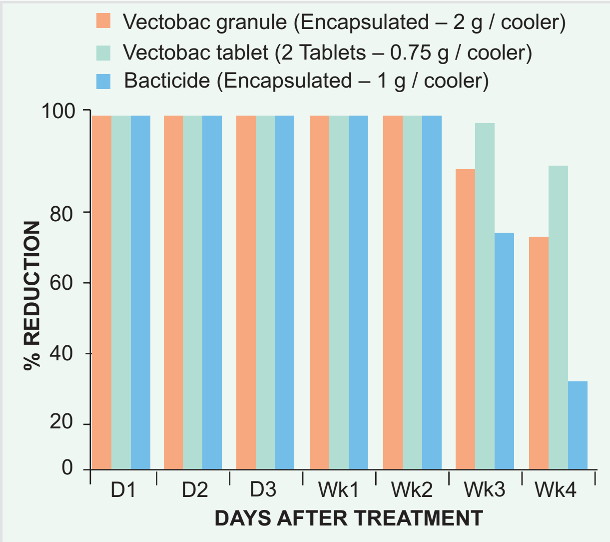

Fig. 71: Efficacy of different formulations in containing *Ae. aegypti* breeding in treated desert coolers

Tacbio

Trial of an Indian strain of *B. thuringiensis* var *israelensis* H14 (*Bti* AS, VCRC B17) against immatures of *An. culicifacies, An. stephensi, Cx. quinquefasciatus* and *Aedes* sp showed 100% mortality up to 100 µl dose and 70–90% mortality at lowest dosages of 10 to 50 µl of biolarvicide. The lowest field application dose of 0.5 ml/m^2 remains effective for about 10–12 days in clean unpolluted water bodies, which support anopheline breeding. However, in stagnant polluted waters such as drains and cesspools supporting culicine breeding, the same dose persists for 5–6 days only. Based on the field observations, an operational dose of 0.5 ml/m^2 at fortnightly intervals is required for clean water sources supporting anopheline breeding. However, to control breeding of culicine mosquitoes in stagnant and polluted waters, an operational dose of 1 ml/m^2 at fortnightly intervals was suggested.

Biolarvicides (*Bti* AS, VCRC B17 strains) were evaluated for their efficacy against immatures of *Anopheles* and *Culex* species larvae in natural habitats and their persistence was assessed. Studies were carried out in Rourkela Municipal area with a control urban area 10 km away from the experimental area. Field application was done with standard equipment following the NVBDCP guidelines for spray. Percent reduction was estimated on subsequent days to determine the efficacy. Impact on adult densities was also determined. Application was done @ 0.5 ml/m^2 in clean waters and @ 1 ml/m^2 in polluted waters. Study was carried out for one year.

The product was found effective for 10–12 days at an application dose of 0.5 ml/m^2 (5 litres per hectare) in clean water and 1 ml/m^2 (10 litres per hectare) in polluted waters with impact on adult densities.These results have shown that this new strain of bacterial larvicide is effective for use in larval control.

One year trial was completed on an Indian strain of *B. thuringiensis* (*Bti* AS, VCRC B17) being manufactured by Tuticorin Alkali Chemicals and Fertilizers and marketed by Godrej Hi-Care under the brand name of Tacbio. The objectives of the trial were: to evaluate the efficacy against mosquito larvae in different natural habitats, impact on adult mosquito density, persistence of biolarvicide in different breeding habitats and to assess the operational dose and its frequency of use. Field application with Tacbio showed that it is effective against immatures of anopheline and culicine mosquito species in different breeding habitats commonly encountered in urban areas. The application of Tacbio resulted in significant reduction in the larval density of III and IV instars ranging from 73–100% in different breeding habitats. (Figs. 72 and 73).

The persistence of Tacbio varies in different breeding habitats. The lowest field application dose of 0.5 ml/m^2 remains effective for about 10–12 days in clean unpolluted water bodies, which support anopheline breeding. However, in stagnant polluted waters such as drains and cesspools supporting

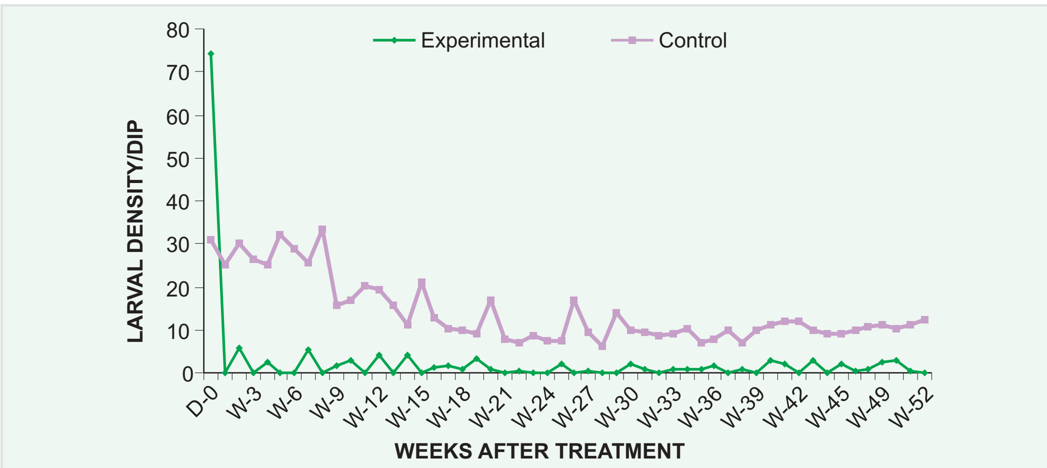

Fig. 72: Impact of Tacbio on the anopheline larval density in cement tanks applied @ 0.5 ml/m^2

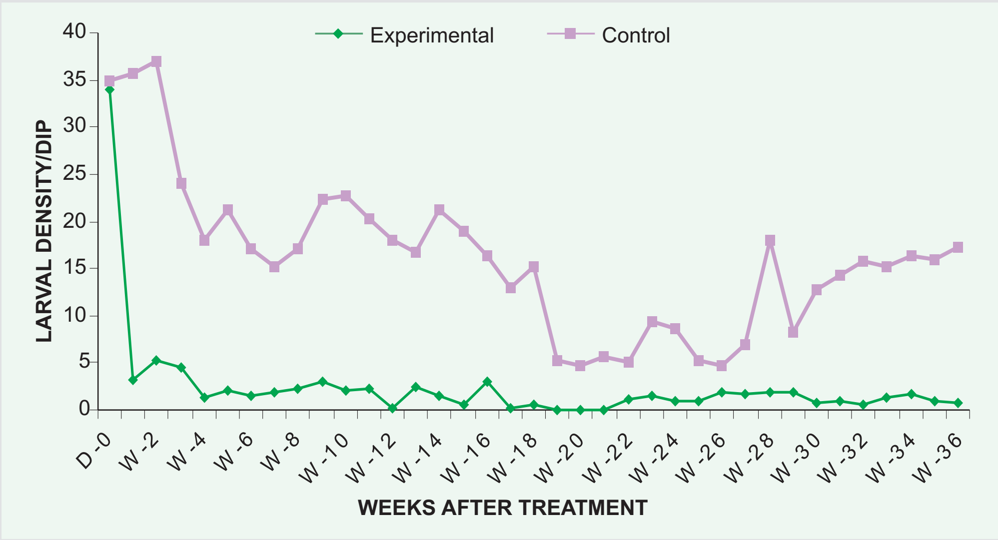

Fig. 73: Impact of Tacbio on the *Cx. quinquefasciatus* larval density in stagnant drains

culicine breeding, the same dose persists for 5–6 days only. A dose of 1 ml/m^2 in stagnant polluted waters was found to be effective for about 10–12 days in controlling culicine breeding. During intervention phase, the overall breeding positivity rate had come down to 15% as compared to 76% in the control area. There was a reduction of 69–86% in the adult mosquito density in comparison to control area.

VectoBac

VectoBac 12AS is a liquid formulation of *Bti* and was also found effective in the control of *An. stephensi, Ae. aegypti, Cx. quinquefasciatus* mosquitoes in a field trial. It was found operationally very feasible to apply in the field. Two more formulations of *Bti*, namely VectoBac WDG and VectoBac tablets, produced by M/s. Valent Bioscience U.S.A and supplied by M/s. Sumitomo Chemicals have been tested in the natural breeding habitats of different mosquito species in different areas. VectoBac WDG formulation was found to be effective against different mosquitoes @ 0.1 to 0.5 g/m^2 for one to three weeks in different habitats. Large scale trials have shown its efficacy @ 0.1 to 0.2 g/m^2 in clear and polluted water habitats when applied at fortnightly intervals.

Most recently a phase III trial of VectoBac WDG was conducted in Ahmedabad City to evaluate its efficacy and persistence against *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus* in intradomestic and peridomestic mosquito breeding habitats. It was applied at the dose of 1.5 kg/ha in clear water (equivalent to 150 mg/m^2 area or 150 mg/100 litre water volume of containers) and 3 kg/ha in polluted water or peridomestic habitats (equivalent to 300 mg/m^2 area). Each application of VectoBac WDG was found to give effective control of larvae/pupae (70–100% mortality) up to three weeks in intradomestic containers and up to two weeks in peridomestic habitats (mortality, 80–100%). After two weeks of VectoBac WDG application, gradual declination in ovitrap positivity in treated area indicates reduction in prevalence of *Ae. aegypti*. For operational reasons, therefore, blanket spraying of VectoBac WDG every two weeks was found to produce effective control of mosquitoes in all kinds of habitats in urban and peri-urban settings.

Bacticide DT

A multicentric trial of Bacticide DT formulation was carried out in urban areas to see persistence in domestic and peridomestic breeding containers, such as desert coolers, containers, water tanks which are potential mosquito breeding habitats of *Ae. aegypti* and *An. stephensi* and also against *Cx. quinquefasciatus.* The study was carried out in urban/periurban areas of Raipur (Chhattisgarh), Hardwar (Uttarakhand) and Sonepat (Haryana) areas. The methodology was based on the common protocol developed by NIMR for evaluation of biolarvicide. The Bacticide DT (400 mg) was evaluated in natural breeding habitats of *Anopheles, Culex* and *Aedes* species. Application of one dispersible tablet (400

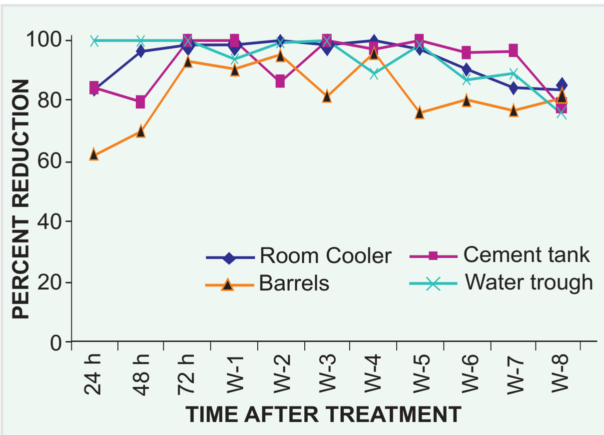

Fig. 74: Effect of Bacticide DT 400 mg on late instars of *Ae. aegypti* in different breeding habitats in Raipur

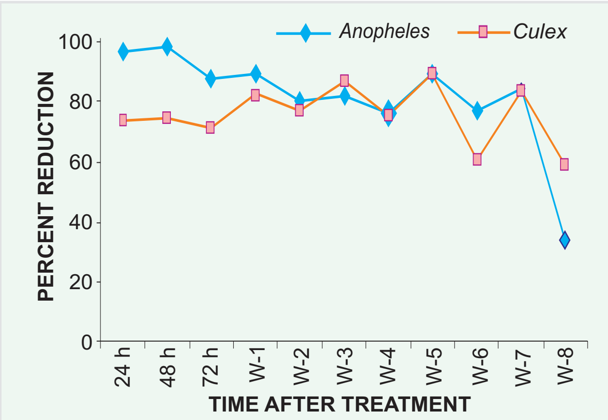

Fig. 75: Effect of Bacticide DT 400 mg on late instars of *An. stephensi* and *Cx. quinquefasciatus* in different breeding habitats in Sonepat

mg)/m² of water surface or one tablet per container with <50 litre of water produced >80% reduction of late instars up to two weeks . In container habitats of *Anopheles* and *Aedes*, where water was >20 litre, the effect was up to four weeks (>80% reduction) (Figs. 74–76). Bacticide DT formulation was more effective against *Aedes* compared to *Anopheles* and *Culex* species.

Bacticide WP

This study was carried out to evaluate the effectiveness of Bacticide WP formulation for control of *An. culicifacies, An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus* in a locality as a multicentric trial. Bacticide WP was evaluated at the dose of 200 mg/m² in natural breeding habitats against *An. stephensi*, *An. subpictus*, *Cx. quinquefasciatus* at Raipur and Sonepat. It was also evaluated against *Ae. aegypti* in Raipur. In Mathura, it was evaluated against *An. culicifacies* and also against *Cx. quinquefasciatus*.

Results showed within a week maximum of 100 percent reduction of late instar larvae of target species *An. stephensi* in coolers, cemented tanks, *An. subpictus* in pits and pools, cemented tanks, clean water drains and *Ae. aegypti* in coolers during post-treatment period. Bacticide WP was effective (>80% reduction) in clean water small habitats for two weeks. (Figs. 77–79). Against *Cx. quinquefasciatus* in surface drains with organic matter, the reduction was >80% for seven days and in small containers such as coolers, tanks the effect was (>80%) for two weeks. In general, the formulation was more effective against *Aedes* compared to *Anopheles* and *Culex*. ❑

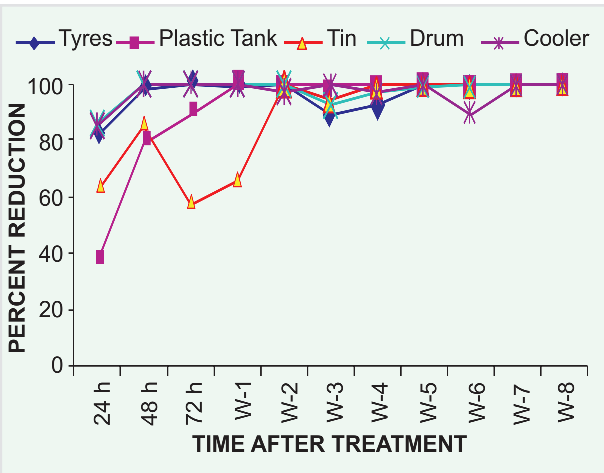

Fig. 76: Effect of Bacticide DT 400 mg on late instars of *Ae. aegypti* in different breeding habitats in Hardwar

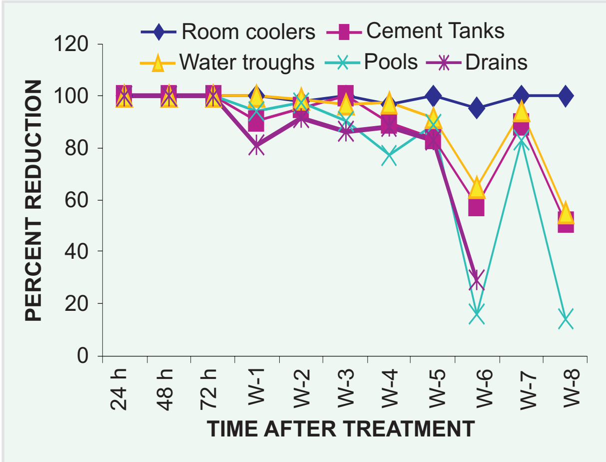

Fig. 77: Effect of Bacticide WP @ 200 mg/m² on late instars of anophelines in Raipur

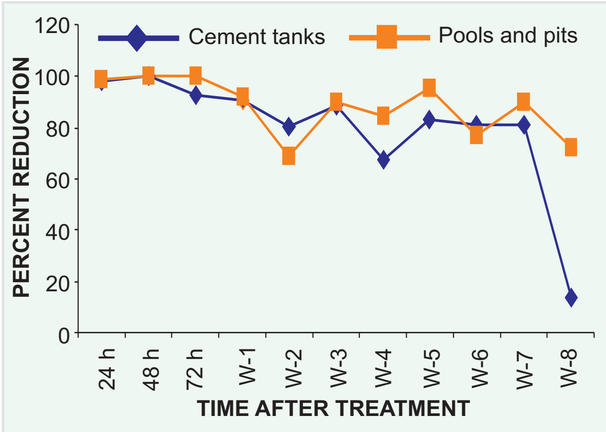

Fig. 78: Effect of Bacticide WP @ 200 mg/m² on late instars of anophelines in Sonepat

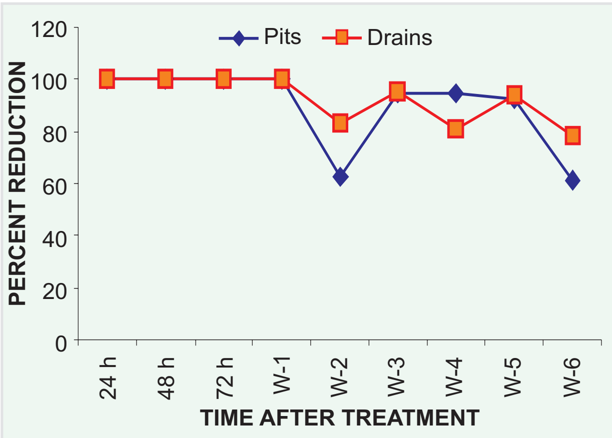

Fig. 79: Effect of Bacticide WP @ 200 mg/m² on late instars of anophelines in Mathura