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# FIELD TRIALS ON THE USE OF *BACILLUS THURINGIENSIS* SEROTYPE H-14 AGAINST *MANSONIA* MOSQUITOES IN MALAYSIA[1]

ANNIE E. S. FOO AND H. H. YAP

School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia

ABSTRACT. A suspension concentrate of *Bacillus thuringiensis* serotype H-14 formulation (Teknar®, SAN 402–1 SC) was tested against laboratory reared late third/early fourth instar larvae of *Mansonia uniformis* as well as naturally occurring *Mansonia* larvae using Hudson knapsack sprayers on small plots in swampy ditches on Penang Island, Malaysia. Six dosages ranging from 1.1 to 11.40 kg/hectare were used in two experiments. Mean dosage/response values at the 50% level for the introduced and natural population were 0.66 and 1.19 kg/hectare, respectively, whereas, the mean dosage/response values at 95% level were11.02 and 25.98 kg/hectare for the introduced and natural population, respectively. Higher dosages of the *B. thuringiensis* H-14 formulation were needed to achieve control of the *Mansonia* larvae when compared with other vector mosquitoes. The heterogeneity of the response of *Mansonia* population towards *B. thuringiensis* H-14 was also observed. The comparable dosage/response values for introduced and natural populations suggest that caged introduced populations can be used as a bioassay method for *Mansonia* larvae in the field.

## INTRODUCTION

There has been a general lack of information concerning larvicidal tests against *Mansonia* using either conventional insecticides or biological control agents. Laboratory tests on the susceptibility of *Mansonia* larvae using conventional insecticides were conducted earlier (Yap et al. 1968, Yap and Sulaiman 1976). Among the biological control agents, *Bacillus thuringiensis* serotype H-14 (de Barjac 1978) has been shown to be a highly effective biological insecticide against vector mosquitoes and simuliid blackflies (Anonymous 1981, 1982). To date, most reports on laboratory and field evaluations of *B. thuringiensis* H-14 have involved *Aedes, Culex* and *Anopheles* mosquitoes. In a comparative laboratory study, the susceptibility of *Ma. indiana* Edwards and other mosquito larvae to a few formulations of *B. thuringiensis*

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H-14 was recently reported (Foo and Yap 1982). In general, *Mansonia* larvae appear to be more tolerant than other vector mosquitoes to insecticides or biocontrol agents.

We are reporting the results of field trials of *B. thuringiensis* H-14 against *Mansonia* larvae on Penang Island, Malaysia. Procedures for field testing of *Mansonia* mosquitoes are also described.

## MATERIALS AND METHODS

Field trials were conducted during November and December 1982 in the swampy ditches of an abandoned coconut plantation in Permatang Damar Laut on the southern coastal alluvial plain of Penang Island, Malaysia. The plots were covered with water hyacinth plants (*Eichhornia crassipes* Solm) and *Mansonia* larvae were present. Six treated and three untreated control plots were used. The nine plots totalled 145 $m^2$ with plot sizes ranging from 15 to 18 $m^2$. The water depth of the plots varied from 45 to 90 cm. Air and water temperatures, daily rainfall, total suspended matter and pH of the field water were measured.

Two separate trials were conducted. In the first trial, the efficacy of the *B. thuringiensis* H-14 Teknar® formulation was studied with laboratory reared late third/early fourth instar larvae of *Ma. uniformis* (Theobald) in screened cages. In the second trial, in addition to the caged introduced mosquitoes, naturally occurring *Mansonia* larvae attached to the water hyacinth plants in the plots were also assayed. The laboratory-reared *Ma. uniformis* larvae were from a stock colony (F-13 generation) established from Penang Island. One hundred late third/early fourth instar *Ma. uniformis* larvae (13 to 15 days old) were placed in each cage in the test plots. The cage (30 × 30 × 15 cm) was constructed of brass screen (Gauge S.W.G. 35, Mesh no. 30) with wooden frames and attached floats. The floats were used to prevent submergence of the cages. Three or four young water hyacinth plants, which were thoroughly cleaned, were placed in each cage to provide attachment for the introduced *Mansonia* larvae. Three cages were placed in each plot prior to treatment. One cage was removed at 24, 48 and 96 hr posttreatment from each plot. Additional cages were introduced at 24 and 48 hr posttreatment to detect the residual effects of the Teknar formulation. Recovery of the introduced larvae was recorded at prescribed intervals.

The pre- and posttreatment sampling of the endemic *Mansonia* population in the swampy ditches was conducted by shaking 20 *Eichhornia crassipes* plants from each plot in buckets to dislodge the *Mansonia* larvae attached to the roots of the plants. The dislodged larvae were then sorted out from the debris. Three pretreatment counts and one posttreatment count were recorded for each of the untreated and treated plots. The three pretreatment counts were conducted within 7 days prior to *Bacillus* spraying. Posttreatment counts were recorded at 24, 48 and 96 hr after spraying, respectively.

The *B. thuringiensis* H-14 formulation was a suspension concentrate of Teknar® containing 600 ITU/mg (SAN 402–1 SC, Lot No. 21331) as provided by Sandoz Ltd., Basle, Switzerland. The formulation was tested at dosages of 1.1, 5.7, 11.4, 28.5, 57.0 and 114.0 kg per surface hectare of the treated plots. One plot was used per dosage whereas three plots were used as controls (untreated). For each dosage, the Teknar formulation was diluted with 4.5 liters of seasoned tap water and sprayed with a 9-liter Hudson knapsack sprayer equipped with lance and nozzle (TEE JET, HSS 8002E). Statistical analysis, including probit analysis (Finney 1962) assisted by use of the computer program of Daum (1970), was employed on the data collected.

## RESULTS

The physico-chemical conditions of the small plots in swampy ditches in Permatang Damar Laut, Penang Island, Malaysia, are given in Table 1. The water temperature immediately after spraying was 33 °C and 25 °C for the first and second trials, respectively. Turbidity of the water was recorded with absorbence value of 0.071 when the water was read at wavelength 415 mm with slit width of 0.034 mm using a Beckman Spectrophotometer. Water depth of the small lots was estimated to range from 45 to

Table 1. Physico-chemical conditions of the small plots in swampy ditches over a 5-day period for trials 1 and 2, respectively.

| Trial no. | Daily temperature (°C) (mean ± S.D.) Air | Water | Total rainfall (cm) | pH | Total suspended matter (mg/liter) |
|---|---|---|---|---|---|
| 1 | 31.1 ± 2.4 | 27.8 ± 2.8 | 83.7 | 6.7 | 13.2 |
| 2 | 29.0 ± 0.9 | 25.3 ± 0.4 | 7.2 | 6.8 | 13.2 |

90 cm. General physico-chemical conditions of Penang Island, Malaysia, were reported earlier (Yap and Ho 1977).

The larvicidal effects of the *B. thuringiensis* H-14 Teknar formulation, against both the introduced caged population of *Ma. uniformis* and the natural *Mansonia* population attached to water hyacinth plants over a 96 hr period, are presented in Table 2. The results indicated a lower recovery of introduced *Ma. uniformis* larvae with longer exposure times. There were substantial reductions in the recovery of natural populations at all dosages when pretreatment and posttreatment counts were compared for each plot.

When treated with probit analysis (Finney 1962) using the computer program (Daum 1970), the above bioassay data provided dosage—response values with linear regression and regression slopes (Table 3). For the introduced *Mansonia* populations for both trials 1 and 2, the $LD_{50}$ values indicated a decreasing trend. This trend was not observed for the natural population. Moreover, the regression slopes for both the natural and introduced population were all very flat with values ranging from 0.70 to 2.70 (Table 3).

When *Ma. uniformis* larvae were introduced at 24 and 48 hr after treatment for an exposure period of 24 hr, there appeared to be no statistically detectable residual effect (F-test; $\alpha = 0.05$) of *B. thuringiensis* Teknar formulation (Table 4).

## DISCUSSION

Results from trials 1 and 2 where introduced *Ma. uniformis* larvae were used in screened cages indicated differences in dosage/response values to the Teknar formulation (Tables 2 and 3).

The dosage of Teknar formulation used in the study ranged from 1.1 to 114.0 kg/hectare (Table 2). Dosages of 5.7 kg/hectare and upward caused distinctive reduction in the introduced as well as natural populations of the *Mansonia* larvae in the small plot trials. The effective dosages for *Mansonia* larvae are higher than for other mosquito species tested (Hembree et al. 1980; Anonymous 1981, 1982; Wraight et al. 1981, 1982; Mulla et al. 1982; Ramoska et al. 1982). The higher effective dosages needed for *Mansonia* control may be due to the presence of water hyacinth plants, the inac-

Table 2. Larvicidal effects of *Bacillus thuringiensis* H-14 Teknar formulation (SAN 402 1 SC) against *Mansonia* larvae in small plots.

| Mosquito population | Treatment received (kg/hectare) | Recovery of larvae | | | |
|---|---|---|---|---|---|
| | | 0 hr[a] | 24 hr | 48 hr | 96 hr |
| | *Trial 1* | | | | |
| Introduced population of 100 larvae per cage | Untreated[b] | — | 78 | 74 | 51 |
| | 1.1 | — | 79 | 74 | 53 |
| | 5.7 | — | 36 | 23 | 3 |
| | 11.4 | — | 30 | 27 | 10 |
| | 28.5 | — | 8 | 8 | 2 |
| | 57.0 | — | 0 | 4 | 0 |
| | 114.0 | — | 0 | 0 | 0 |
| | *Trial 2* | | | | |
| Introduced population of 100 larvae per cage | Untreated[b] | — | 60 | 60 | 67 |
| | 1.1 | — | 39 | 14 | 4 |
| | 5.7 | — | 0 | 0 | 5 |
| | 11.4 | — | 0 | 1 | 2 |
| | 28.5 | — | 1 | 0 | 0 |
| | 57.0 | — | 0 | 2 | 0 |
| | 114.0 | — | 0 | 1 | 0 |
| | *Trial 2* | | | | |
| Natural population from 20 *Eichhornia* plants | Untreated[b] | 32 | 32 | 14 | 15 |
| | 1.1 | 19 | 11 | 8 | 13 |
| | 5.7 | 25 | 6 | 5 | 4 |
| | 11.4 | 28 | 0 | 5 | 0 |
| | 28.5 | 29 | 0 | 4 | 0 |
| | 57.0 | 39 | 0 | 0 | 0 |
| | 114.0 | 21 | 1 | 0 | 0 |

[a] Means of 3 pretreatment counts.
[b] Means of 3 untreated plots.

cessibility of the *Mansonia* larvae due to their peculiar habitats of attaching to the roots of plants, as well as other tropical environmental factors.

Dosage/response values obtained from probit analysis of the data showed that the larvae succumbed to decreasing dosages over the 96 hr exposure period (Table 3). This may indicate general weakening of the introduced *Mansonia* larvae in the cages due to the Teknar formulation. Other environmental parameters may act on weakened larvae, hence less recovery of live larvae over the longer exposure time. This increased susceptibility was not observed for the natural *Mansonia* populations (Table 3).

Regression slopes of the dosage response regression lines from these field trials were all very flat (Table 3). This is in accordance with

Table 3. Dosage/response values (kg/hectare) for *Bacillus thuringiensis* H-14 Teknar formulation against *Mansonia* larvae in small plots.

| Mosquito population | Post-treatment (hour) | Lethal dosage (kg/hectares) | | | | |
|---|---|---|---|---|---|---|
| | | $LD_{50}$ | 95% C.L. | $LD_{95}$ | 95% C.L. | Slope ± S.E. |
| | | *Trial 1* | | | | |
| Introduced population | 24 | 6.62 | 5.97–7.25 | 36.13 | 32.05–41.50 | 2.23 ± 0.10 |
| | 48 | 5.34 | 3.69–7.07 | 50.89 | 36.06–83.31 | 1.68 ± 0.14 |
| | 96 | 2.94 | 1.42–4.44 | 17.41 | 11.50–36.35 | 2.13 ± 0.30 |
| | Mean[a] | 5.01 | 3.63–6.40 | 34.84 | 26.28–51.69 | 1.95 ± 0.15 |
| | | *Trial 2* | | | | |
| Introduced population | 24 | 1.35 | 90.2–3.12 | 5.51 | 2.50–3349.98 | 2.70 ± 0.85 |
| | 48 | 0.08 | 0.01–0.38 | 7.14 | 3.40– 22.07 | 0.84 ± 0.16 |
| | 96 | 0.02 | 0.01–0.05 | 3.43 | 2.19– 5.07 | 0.70 ± 0.10 |
| | Mean[a] | 0.66 | 0.17–1.30 | 11.02 | 6.45– 27.31 | 1.34 ± 0.19 |
| | | *Trial 2* | | | | |
| Introduced population | 24 | 1.18 | 0.27–2.38 | 21.65 | 10.80– 91.03 | 1.30 ± 0.21 |
| | 48 | 0.75 | 0.34–1.27 | 76.45 | 46.45–157.14 | 0.82 ± 0.09 |
| | 96 | 1.64 | 1.31–1.98 | 9.36 | 7.38– 12.72 | 2.17 ± 0.19 |
| | Mean[a] | 1.19 | 0.94–1.45 | 25.98 | 21.30– 32.77 | 1.23 ± 0.07 |

[a] Dosage/response values based on combined data of 3 posttreatment counts at different intervals.

Table 4. Residual effects of *Bacillus thuringiensis* H-14 Teknar formulation (SAN 402-1 SC) against *Mansonia uniformis* larvae in cages in small plots, introduced at 24 or at 48 hr posttreatment and exposed in the plot for 24 hr.[a]

| Mosquito population | Treatment received (kg/hectare) | Posttreatment recovery of larvae | |
|---|---|---|---|
| | | 24–48 hr | 48–72 hr |
| | *Trial 1* | | |
| Introduced population of 100 larvae per cage | Untreated[b] | 74 | 84 |
| | 1.1 | 88 | 95 |
| | 5.7 | 78 | 97 |
| | 11.4 | 67 | 87 |
| | 28.5 | 73 | 88 |
| | 57.0 | 52 | 95 |
| | 114.0 | 48 | 89 |
| | *Trial 2* | | |
| Introduced population of 100 larvae per cage | Untreated[b] | 67 | 77 |
| | 1.1 | 71 | 85 |
| | 5.7 | 64 | 81 |
| | 11.4 | 39 | 85 |
| | 28.5 | 55 | 90 |
| | 57.0 | 74 | 65 |
| | 114.0 | 35 | 85 |

[a] F-tests computated for the 24 hr and 48 hr posttreatment residual effects were found to be non-significant at $\alpha = 0.05$ for both trials.

[b] Means of 3 untreated plots.

laboratory tests on insecticides (Yap et al. 1968, Yap and Sulaiman 1976) and *B. thuringiensis* formulations (Foo and Yap 1982). The small slope values may indicate the heterogeneity of the *Mansonia* populations towards insecticides and biocontrol agents.

No residual effect was detected by this bioassay method at 24 hr posttreatment (F-test at $\alpha = 0.05$). This is in contrast to the slightly longer residual effects of *B. thuringiensis* formulations carried out in a temperate environment (Mulla et al. 1982).

In general, the control of *Mansonia* has so far been confined to the use of adulticides and some source reduction methods such as removal of host plants by herbicides (Chow 1953, 1957). This field study presented a workable field bioassay method for assessing the effects of toxicants against *Mansonia* larvae. In the present study, the dosages for *Mansonia* control may not be considered practical or cost-effective. However, research into new formulations such as floating slow-release formulations or encapsulations of biological agents and conventional insecticides may provide more suitable methods for the control of *Mansonia* immatures.

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