RANDOMISED, CONTROLLED, BLINDED FIELD TRIAL ON THE EFFICACY OF BIOCIDE FORMULATION (*BACILLUS* SPP.) IN THE CONTROL OF MOSQUITO VECTORS

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ABSTRACT

A randomised, controlled, blinded field trial was conducted in a large cantonment area, to study the efficacy of the biocide formulation (*Bacillus* spp.) indigenously produced by Central Drug Research Institute, Lucknow, India, as a mosquito larvicide, in comparison with Fenthion and Temephos. The study revealed that the biocide formulation in dosage of 5 grams per square metre brought about a very high level of control of culicine larvae from third day of application and the effect persisted till at least 28 days (median values of percentage reduction achieved being 93% to 100%). This effect was statistically significant from 7th to 28th day, as compared to Fenthion, Temephos or the biocide in dosage of 2 grams per square metre. Fenthion, on the other hand was statistically more efficacious in bringing about a quick reduction, with a 100% reduction being obtained within a day, but the effect declined to a low level by 7th day.

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KEY WORDS : Mosquito control; Biological pest control; Randomized control trial.

Introduction

It is almost a century since Sir Ronald Ross & Manson described the epidemiology of malaria and filariasis [1]. Their illustrious discoveries initiated a herculean effort - man's quest to conquer these diseases, which even today remains a distant dream. The unending list of various mosquito control agents, including the "recently introduced insecticides" in use in Armed Forces indicates that we are still nowhere near the perfect insecticide. There is, therefore, an imperative need to develop alternate methods of vector control. In this context, biological agents offer many advantages over chemicals, viz, high specificity, biodegradability, safety to environment, low cost, recycling properties, and prolonged action, which make the biocides an important and useful tool in Integrated Vector Control programmes, particularly in context of Indian Armed Forces who have

always accorded a high priority to control of mosquito borne diseases.

An earlier field trial carried out by Bhalwar et al in 1991 had demonstrated the efficacy of *Bacillus sphaericus* as a mosquito larvicidal agent [2]. Since then, biocide formulation has been produced in our country by Central Drug Research Institute (CDRI) Lucknow. The present trial was therefore undertaken to evaluate the efficacy of this indigenous product under the field conditions.

Material and Methods

The study was undertaken in Lucknow Cantonment, which is a prototype of a typical large cantonment in India. It has well established, ongoing mosquito control measures. Preliminary planning and pilot study was undertaken between 16 August to 25 September, while the actual study was undertaken from 29 September to 3 November 1993,

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i.e. during immediate post-monsoon period when environmental conditions are conducive to mosquito breeding.

The major mosquito breeding points were indentified from the updated spot map, and numbered serially. Out of these, five points were randomly selected for study. They were all open ponds, with circinate margins, showing pollution, moderate vegetation and absence of aquatic fauna; the surface area varied from 80 to 120 square metres. No insecticide was sprayed on these 5 selected points from 4 weeks before start of actual study. All the study points showed exclusively culicine larvae. Sample size was calculated based on the available data which indicated that on 28th day post treatment with currently used insecticides, the mean larval count (III and IV instars) was likely to be 15 per dip with a standard deviation of 2. Keeping an alpha error of 0.05 (two tailed), and beta error of 0.20, the minimum sample worked out to be 2 dips per study area. This was further increased to 10 dips per study area since inter-group comparisons were planned, with a view to improve the precision.

The biocide used in the present study was formulated from *Bacillus* spp., which is a water dispersible powder prepared by Central Drug Research Institute (CDRI), Lucknow.

Two doses were used as done by other workers - 2 grams and 5 grams per square metre [3]. The 5 randomly selected study areas were randomized using lottery system into (i) a control area (no insecticide applied); (ii) biocide 2 g per sq mt; (iii) biocide 5 g per sq mt; (iv) Fenthion 0.05% solution in water and (v) Temephos 0.0125%. Blinding was ensured, in that the principal worker who was evaluating the outcome (larval densities) was himself unaware as to which particular area has been treated with which insecticide, the codes being handled by one of the co-workers.

Percent reduction in (third and fourth instar) larvae was calculated using the following formula which has been used by various other workers [2-5].

$$\text{Reduction} = 100 - (C1/T1 * T2/C2 * 100),$$

where :-

C1 =	number of larvae in pre-treatment control area
C2 =	number of larvae in post-treatment control area
T1 =	number of larvae in pre-treatment treated area
T2 =	number of larvae in post-treatment treated area

The statistical issues have been subjected to Kruskal-Wallis test for overall testing followed by Mann-Whitney 'U' test for "between the individual groups" comparisons after duly correcting the alpha error for multiple group comparisons [7]. During the period of study, the average values of maximum and minimum temperatures (in celsius) were 30.2 and 20.3 respectively, while the relative humidity was 65% and rainfall was nil.

Results

Overall levels of controls achieved : The median values of percentage reductions achieved by the various regimens at different time periods are presented in Table 1 and Fig 1. It is seen that Fenthion achieved a complete control (100%) within 24 hours and the effect was maintained till 72 hours, dropping down thereafter to low levels from 7th day onwards. On the other hand, biocide in the dose of 5 grams achieved control more slowly, from approximately 86% reduction in larval density in 24 hours to 100% control on 14th day. This efficient control was maintained for a longer period, being as high as 93% even on the 28th day. The reductions achieved with the other two treatment modalities, viz, Temephos and biocide 2 grams were, however, found to be much less effective all through the period of study.

Efficacy at 24, 48 and 72 hours : The in-between comparisons of various treatment modalities, according to various time periods are presented in Table 2. It is seen that at 24, 48 and 72 hours, Fenthion was clearly the best

Fig. 1 : Comparison between efficacy of Fenthion, Temephos and biocide.

TABLE 1

Median values of percentage reductions in larval densities according to different time periods

Treatment group	Median values of percentage reductions in larval densities (all figures in %)							
	Hours			Days				
	24	48	72	7	14	21	28	35
Biocide 5 g	85.9	87.1	97.0	90.5	100	100	93.3	77.1
Biocide 2 g	85.6	13.9	-13	-19.5	18.5	49.5	52.5	40.4
Fenthion	100	100	100	33.1	23.5	40.0	51.7	25.1
Temephos	48.3	70.5	88.5	-5.0	23.0	24.9	-8.6	-1.5

as compared to other treatments. The higher median levels of control with Fenthion were found to be statistically significant during this period. Biocide 5 grams also showed higher levels of control as compared to biocide 2 grams and Temephos. But in general, these higher levels were not statistically significant during the period of first 72 hours.

Efficacy after 7, 14, 21, 28 and 35 days after treatment : The results are summarised in Table 3. It was seen that on the 14th day,

Biocide 5 g had maintained a very high effect with its median value of 100% reduction being statistically significantly higher than the median values achieved by biocide 2 g (18.5%), Fenthion (23.5%) or Temephos (23%), while the other three treatments (biocide 2g, Fenthion, Temephos) did not show any significant difference amongst each other.

This statistically significant superior effect of biocide formulation 5 g dose was

TABLE 2

Comparison between the efficacies of different insecticides at 24, 48 and 72 hours after application

Comparison group	Difference in the median values of % reductions (p values in brackets)		
	24 hrs	48 hrs	72 hrs
Fenthion vs biocide 2 g	14.4 (< 0.05)	86.1 (< 0.05)	113 (< 0.05)
Fenthion vs biocide 5 g	14.1 (< 0.01)	12.9 (< 0.05)	3.0 (< 0.05)
Fenthion vs Temephos	51.7 (< 0.05)	29.5 (< 0.05)	11.5 (< 0.05)
Biocide 5 g vs biocide 2 g	0.3 (> 0.05)	73.2 (< 0.05)	110 (< 0.05)
Biocide 5 g vs Temephos	37.6 (> 0.05)	16.5 (> 0.05)	8.5 (< 0.05)
Temephos vs biocide 2 g	37.3 (> 0.05)	56.6 (< 0.05)	101 (> 0.05)

TABLE 3

Comparison between the efficacies of different insecticides from 7th to 35th day after application

Comparison group	Difference in the median values of % reductions (p-values in brackets)				
	Days after application				
	7	14	21	28	35
Biocide 5 g vs Fenthion	42 (< 0.05)	76.5 (< 0.05)	60 (< 0.05)	41.6 (< 0.05)	52 (> 0.05)
Biocide 2 g vs biocide 5g	101.5 (< 0.05)	81.5 (< 0.05)	50.5 (< 0.05)	40.8 (< 0.05)	36.7 (> 0.05)
Biocide 5 g vs Temephos	88.5 (< 0.05)	77.0 (< 0.05)	75.1 (< 0.05)	101.9 (< 0.05)	78.6 (> 0.05)
Fenthion vs biocide 2 g	40.0 (> 0.05)	5.0 (> 0.05)	-9.5 (> 0.05)	-0.8 (> 0.05)	-15.3 (> 0.05)
Fenthion vs Temephos	8.0 (> 0.05)	0.5 (> 0.05)	15.1 (> 0.05)	60.3 (> 0.05)	26.6 (> 0.05)
Temephos vs biocide 2 g	20.0 (> 0.05)	4.5 (> 0.05)	24.6 (> 0.05)	61.1 (> 0.05)	41.9 (> 0.05)

maintained at 21st day (median reduction 100%) and 28th day (median reduction 93.29%).

However, on the 35th day, the percentage reductions achieved by all the 4 forms of treatment had come down to a low level and moreover, there was no statistically significant difference among any two of these treatments. Thus, under the field conditions of the present study, the (statistically significant) superior effect of biocide formulation in doses of 5 g per square metre waned away by the 35th day.

Discussion

From the above findings, it is apparent that *Bacillus* spp. formulation used in the present trial in dosage of 5 g per square metre is a very effective larvicide against culicine larvae in routine field conditions. A good effect (85% median value of reduction achieved) is obtained within 1 day, but it takes 3 days to obtain a maximum effect (97%). The pro-

longed action is apparent from the observation that a very high level of control (93 to 100%) is maintained till 28 days and during this period, this larvicide is statistically significantly superior to other forms of larvicides (Fenthion, Temephos, biocide 2 grams dose). On the 35th day, however, all the four forms of treatment (including biocide 5 g dosage) do not have any statistical difference and the level of reduction drops down.

Fenthion on the other hand is very effective immediately with a 100% reduction obtained on first day and lasting till 3 days. However, the larvicidal effect drops down to a low level by the 7th day. (Fig 1). Temephos and biocide in 2 g dosage are clearly inferior to biocide 5 g or Fenthion (Fig 1).

The findings of the present study are in general agreement with those of earlier workers [2,3,8-11].

Though the present study has not evaluated the efficacy of this biocide in controlling

Anopheline larvae, the findings of earlier workers indicate that the agent is quite effective against these species also [11].

It is suggested that indigenously produced biocide formulation in dosage of 5 g per square metre may be considered for use in the cantonments, for routine mosquito control operations. A very clear advantage would be that applications would be required once in 28 days (compared to once in 7 days with currently used larvicides), thus greatly saving manpower and effectiveness of operations. The environmental and personal safety would be additional benefits. The use of Fen-thion may be reserved for exigencies when a quick effect is desired (as high mosquito-genic conditions or impending outbreak of mosquito borne diseases), while Temephos may continue to be used for potable water bodies.

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