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Evaluation of the efficacy of bendiocarb 80% WP as indoor residual spray on three local building materials against *Anopheles arabiensis* (Patton) (Diptera: Culicidae) using laboratory models

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Abstract

In the Sudan, the indoor residual spraying (IRS) campaigns currently depend on spraying the carbamate insecticide Bendiocarb (2, 2 dimethyl-2H-1,3-benzodioxol-4-yl methyl carbamate; Ficam® 80% WP) at 200 mg/m² against *Anopheles arabiensis* Patton (Diptera: Culicidae) and other mosquitoes and some household pests. Households building materials in the targeted villages are mainly constructed by using different local materials (wood and straw), mud bricks and a third group use cement in their walls. The residual activity (persistence) of Bendiocarb on the different building substrates/materials (*viz*. straw, mud, cement), and glass, which is used as non-absorbent substrate was determined. The susceptibility of mosquitoes to Bendiocarb was tested using 5 concentrations (i.e. 80, 40, 20, 10, 5, 2.5 ppm). Ten adults were exposed for 24 hr. to each of these concentrations, which was replicated 4x, and the experiment was repeated twice. The substrates were attached to the wall and Bendiocarb was sprayed as recommended by WHO; WHO recommended bioassay tests were carried out for evaluation using standard WHO cones. The LC50 proved to be 58ppm and LC90 143 ppm, and the slope of log-dose-probability line (Ld-P lines) 3.04. During the first months 100% mortality was obtained for the different substrates. The 2nd month tests showed 100% mortality for all substrates, except cement (92.5% mortality). In the 3rd month, the mortality rate was 100% on straw and glass, but 95% and 87.5% mortality were registered on mud and cement, respectively. The 4th month results were 60, 72.5, 80, and 90% mortality on cement, mud, straw and glass, following the same order. However, after 5 months, the mortality was: 25% for cement, 35% for mud, 42% for straw, and 65% for glass. It is concluded that the cement is the substrate in which the Bendiocarb performance was the lowest of the 4 tested materials. Bendiocarb stayed longer in mud building material, followed by the straw. In the glass substrate, Bendiocarb persisted longer than all the other substrates, which could be attributed to its nature as not absorbent and inert material. Therefore, Bendiocarb should be used as an effective IRS insecticide against *A. arabiensis* at 200 mg/m² and for the tested building materials, treatment should be at least 2x / yr.

Keywords: Bendiocarb, ficam ® 80% WP, IRS, *Anopheles arabiensis*, bioassays, cones bioassay test, building material

1. Introduction

Currently, house spraying with residual insecticide is one of the most important advances in insect control, in which the insecticides remains active/effective over extended periods. Spraying with residual insecticide became particularly important in the control of the mosquito (Diptera: Culicidae) vectors of malaria and other diseases, which often rest on walls before and after feeding [1].

When applied properly, indoor residual spraying (IRS) is a powerful malaria vector control intervention, rapidly reducing vector-transmission capacity and malaria incidence. IRS provides maximum mass-effect on the vector populations, when it is applied at high coverage levels [2]. The use of bendiocarb (2, 2-dimethyl-2*H*-1, 3-benzodioxol-4-yl methylcarbamate) as a suitable insecticide to use in IRS in the Sudan was initiated, because of the development of resistance to DDT, Malathion, and recently the pyrethroids. Moreover, Bendiocarb represents the sole insecticide tested against *An. arabiensis* and it showed 98-100%. Mortality in the states of the country.

The use of Bendiocarb began in Gezira State (central Sudan) in 2008, and was scaled up to include the southern neighboring State of Sinnar as a tactic to manage IRS problems there. Initially, the use of Bendiocarb in IRS to control mosquito vectors contributed to suppressing malaria episodes in Gezira and Sinnar States by 50% in general [3].

The interaction between the insecticides and construction substrates on the surfaces of the walls is expected to affect the insecticides activity/ performance. The nature of the substrate (chemical and physical) plays an important role in the persistence of the insecticide. Some studies revealed that the insecticide was most persistent on wood and lime-coated mud surfaces. These surfaces are less porous in nature, avoiding the quick penetration of the insecticide occurring on mud blocks [4-6]. The most rapid loss in insecticide toxicity occurs on mud blocks, indicating strong absorption, adsorption or breakdown of the insecticide by alkaline substances [7, 8]. The lack of insecticide persistence on porous surfaces was noted before [4, 5, 9-12]. Moreover, the notable variability in mortality on porous substrates, have also been noted [8]. Concrete surfaces are also porous substrates and, e.g. the application of corn dust to them causes a decline in the pH and a reduction in the porosity, improving the residual effect of insecticides [7]. Applying commercial sealants to concrete prior to insecticide application formed a water-proof barrier and improved the residual effect of the parathyroid cyfluthrin WP [13]. Lime-coated mud blocks showed improved insecticide efficiency. This was attributed to improved particle adhesion and decreased porosity of the mud [14].

2. Materials and Methods

The study was conducted at The Blue Nile National Institute for Communicable Diseases, University of Gezira, Wad Medani, and the Sudan. The selected building materials were the commonly used materials in the villages, towns and cities of the country, according to the standard of living, *viz*. mud, cement and straw.

2.1 Mosquitoes tested

The larvae were collected monthly from January to May, using standard larval collection procedures employing dippers screened nettings, plastic pipettes, and metal dishes/bowls,pooled in plastic buckets and transferred to the laboratory for identification and rearing in the insectary rearing trays and fed on fish powder. The pupae were collected from the trays using wide-mouthed pipettes, placed in plastic cups containing clean tab- water and placed inside adult cages. Emerging adults were fed on 10% sugar solution and used for the bioassay. For verification of the species, samples from both the larvae and emerged adults' were subjected to morphological identification [15].

2.2 Building material (substrates) preparation

Two sorbent substrates (cement and mud) were prepared with a thickness of 2 cm in 20 cm² frame. The straw (commonly used in cottages and poor people houses) was cut in a manner where the cone could be fitted on it easily.

2.3 Preparation and application of the Bendiocarb suspension

One sachet (62.5 g) of Bendiocarb (Ficam® 80% WP) was dissolved in 10 L of water into the sprayer tank to obtain 200 mg A.I. / m². The prepared substrates were fixed to the wall, and Ficam® 80% WP was applied using standard WHO recommended Hudson pump sprayer, fitted with pressure gauge and HSS-8002 nozzle tips, and equipped with regulator set at 55 PSI pressure. The discharge rate was 760 ml/min (25 m²/L to a point of runoff). The nozzle was positioned 45 cm from the surface to give spray swath 65-70 cm wide. The dried blocks were fixed on to the vertical surfaces of the wall. A total of 20 blocks were sprayed and held in a wooden box away from light and wind at laboratory conditions (25 °C ± 2 °C and 80% ± 10% R.H). Four blocks of each substrate were prepared; one remained as untreated control. Four replicates of each substrate were treated with insecticide.

2.4 Assessment of Mortality

The number of dead adults (treated and control) was taken 24 hr. post-exposure. A mosquito was classified as dead, if it is immobile, or unable to stand, or fly in an uncoordinated way. The mortality was calculated by summing the number of dead mosquitoes across all four replicates and expressed as a% of the total number of exposed mosquitoes:

$$\frac{\text{total number of dead mosquitoes} \times \text{observed mortality}}{\text{total sample size}} \times 100$$

If the control mortality is > 20%, the tests must be discarded. When control mortality is > 5%, but < 20%, then the observed mortality has to be corrected using Abbot's formula, as follows:

$$\frac{(\%\ \text{observed mortality} - \%\ \text{control mortality})}{(100 - \%\ \text{control mortality})} \times 100$$

If the control mortality is < 5%, it can be ignored and no correction is necessary, when reporting mortality counts, the sample size should always be given, and preferably an estimate of the 95% confidence intervals [16].

2.5 Statistical Analysis

The bioassay test results were subjected to Probity analysis (Excel program), and the results of cone bioassay test subjected ANOVA for detecting the differences between treatments, and Duncan´s Multiple Range test for mean separation.

3. Results

3.1 Bioassay

This test was conducted to test the susceptibility of the BNNICD strain to Bendiocarb, and the log-dose probability line (Ld-p line) will be used as a standard curve to measure the concentrations that caused the mortality in the treatments. The regression line equation was Y = 2.084+2.843X. From this line the LC50 and LC90 were calculated (Table 1; Fig.1).

From the Probit analysis, the LC50 proved to be 15 ppm and LC90 was 24 ppm. The slope of the line was 2.08.

3.2 Cone Bioassay Test on Building materials

After 24 hr. of application, all building materials showed 100% mortality. Same results were reported 1 month later. Mud, straw and glass were still giving 100% mortality, whereas the cement efficacy decreased to 92.5% after 2 months from application. Three months after application only the straw and the glass resulted in 100% mortality, whereas the cement effected 87% mortality and the mud 94% mortality. The 4th month tests showed a trend of deterioration in all surfaces. Cement, mud, straw and glass resulted in 60, 72, 80 and 89% mortality, respectively. This trend of deterioration continued in the 5th month and the mortalities reported were 25, 35, 42 and 65%, following the same order (Table 2). From tables 1 and 2, the concentrations during the 5 months were calculated based on the mortalities caused by the residues of Bendiocarb on each surface. Table 3 showed the approximate concentrations for each building material by time. The initial concentration in all surfaces was estimated to be > 27.8 ppm. It continued within that limit up to 30 days in all surfaces. However, in cement the concentrations started to decrease to 15.4, 14.2, 11.1 and 7.9 ppm, respectively for the 2nd – 5th month. For the other three surfaces, the concentration seems to have continued as 27.8 for the 2nd month. During the 3rd month, mud registered 15.7 ppm, whereas the straw and glass were able to show the effects caused by the initial concentration (27.8 ppm). By the end of the experiment (5th month), the estimated concentrations were 8.8, 9.5 and 11.6 for mud, straw and glass, respectively.

Table 1: *An. arabiensis* adults (females) (BNNICD susceptible strain) mortality when exposed to 6 concentrations of Bendiocarb 80% WP using WHO method

Conc. (ppm)	% Mortality	LC50 (ppm)	LC90 (ppm)	Ld-p lines Slope	Regression Line equation
40.0	93	15	24	2.84	Y = 2.084+2.843X
20.0	90				
10.0	33				
5.0	15				
2.5	5				

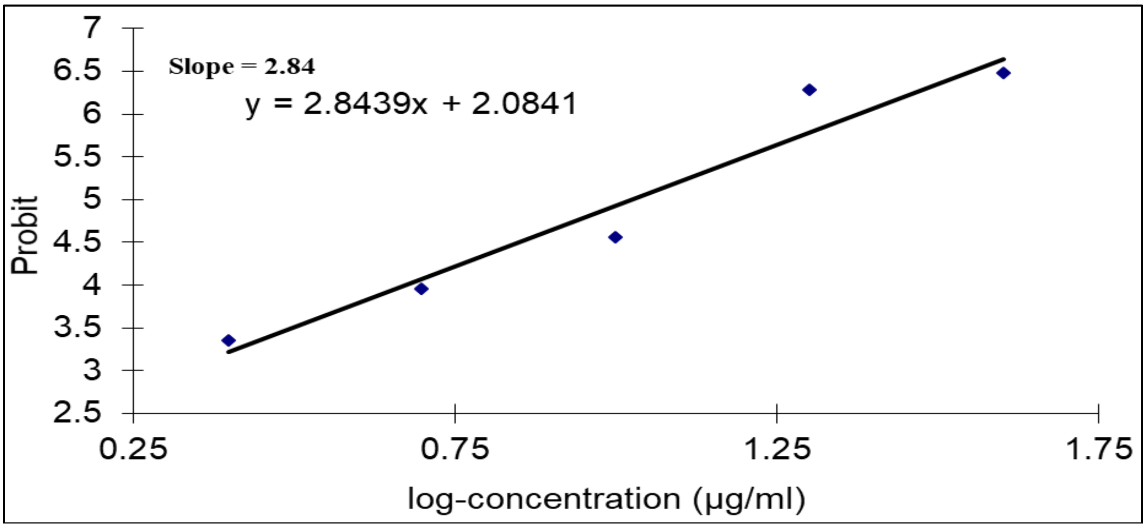

Fig 1: Ld-p line for Bendiocarb using BNNICD susceptible strain of *An. arabiensis* adult females

Table 2: Mortality (%) for *A. Arabiensis* females exposed for 5 months to Bendiocarb 80% - treated building material surfaces at 200 mg/m²

Substrates	After 24 hr.	Months (% mortality)				
		1st	2nd	3rd	4th	5th
Cement	100	100	92.5	87	60	25
Mud	100	100	100	94	72	35
Straw	100	100	100	100	80	42
Glass	100	100	100	100	89	65

Table 3: Concentration (Persistence/ stability) of Bendiocarb (ppm) on tested building materials

Substrates		Month (ppm)				
	24 hr.	1st	2nd	3rd	4th	5th
Cement	27.8	27.8	15.4	14.2	11.1	7.9
Mud	27.8	27.8	27.8	15.7	12.2	8.8
Straw	27.8	27.8	27.8	27.8	13.2	9.5
Glass	27.8	27.8	27.8	27.8	14.5	11.6

4. Discussion

Determination of the residual activity of insecticides is an essential component in the selection of an appropriate insecticide for IRS operations. In a study [17] mosquito mortality rates observed after exposure to the Bendiocarb treated substrates, revealed that the 1st wk after treatment (WAT), 100% mortality was recorded among both susceptible and parathyroid-resistant mosquitoes, in all treated surfaces in the laboratory and the field. In following weeks, the number of dead mosquitoes after exposure to treated surfaces varied considerably, i.e. a decrease in the availability of the Bendiocarb on these surfaces. Bioassay indicated that the residual life of Bendiocarb on red clay walls and its mixture with cement was lower. The authors [17] attributed that to the higher porosity of these substrates than the others. The efficacy of Bendiocarb decreased below 80% on walls made with red clay 5 WAT. This residual life of this insecticide is lower than that reported on mud in India [18], which revealed a persistence of at 100% mortality for about 10 wk on mud, whereas in Zimbabwe [19], the insecticide provided 74% mortality up to 5 months after spray on mud. In Cameroon [20], 13 wk after spray on mud was reported. The estimated efficacy of this insecticide in terms of *An. gambiae S.S.* killing was 80%.

The interaction between insecticide and surfaces resulted in a rapid decrease of its activity. Therefore, the nature of the substrate is expected to play an important role in the decay rate. In some trials [7, 8] the insecticide was most persistent on wood and lime-coated mud surfaces, which are less porous in nature, avoiding the quick penetration of the insecticide occurring on mud blocks [4-6]. The most rapid loss in insecticide toxicity occurs on mud blocks (strong absorption or breakdown by alkaline substances). The lack of insecticide persistence on porous surfaces was reported earlier [4-6, 12]. Moreover, the notable variability in mortality on porous substrates have also been documented by others [8]. Concrete surfaces are also porous and the application of corn dust to them causes a decline in the pH and a reduction in its porosity, improving the residual effect [4]. Applying commercial sealants to concrete prior to insecticide application formed a waterproof barrier and improved residual efficacy of cyfluthrin WP [13]. Lime-coated mud blocks showed improved insecticide efficiency [14]. The authors attributed that to various reasons, *viz.* improved particle adhesion and decreased porosity of the mud.

In Egypt [18], laboratory study evaluating the residual bio-efficacy of 4 insecticides sprayed on the most common house-wall surfaces (wood, mud, and cement) against *Phlebotomus papatasi* (Diptera: Psychodidae) and *Culex pipiens* (Diptera: Culicidae), WHO cone bioassay revealed that effective and extended control (≥ 80% mortality) was produced by lambda-cyhalothrin on indoor wood and cement surfaces. The insecticide effectively controlled (> 80% mortality) *P. papatasi* and *Cx. pipiens* for 10 and 12 wk post-spray on wood surfaces, respectively. Deltamethrin effectively controlled *Cx. pipiens* for 8 wk on indoor wood, mud, and cement surfaces. Indoor and outdoor-kept surfaces treated with permethrin and Malathion provided negligible efficacy against both species. The authors concluded that the surfaces might play inhibiting role in IRS-based vector control endeavors in rural areas in developing countries.

Modified wall surfaces can potentially lead to a substantial increase in the relative risk of mosquito bites [19]. The authors concluded that the intensity of the adjusted impact varies according to the scale of the modification, and use of alternative interventions.

The residual activity of deltamethrin @ 25 mg/m² was studied under laboratory conditions on different surfaces (cement, mud and plaster) (20). Three sorbent surfaces, i.e. cement, mud and plaster were prepared in pans. Wood and filter paper were cut in a situation that bioassay cone could be fitted on them easily. *An. stephensi* showed that filter paper surface have best residual capacity of deltamethrin (67.65% mortality), followed by plaster (64.46%), wood (63.69%), cement (58.4%) and mud (28.28%) after 5 months of IRS application. Bioassay test on *Ae Aegypti* showed almost similar trend. Whole study depicted that residual effect of deltamethrin on different surfaces is variable.

Formerly conducted test in Wad Elshaffie Fallata locality, Gezira State, for the Bendiocarb [21] using an application rate of 240 mg/m² against *An. arabiensis* revealed a residual effect on sprayed surfaces (cement, mud and wood) of 4 months. The present study bioassays test on the same vector revealed that Bendiocarb, resulted in 100% mortality for the different substrates during the first two months, except cement (92.5% mortality). Then the mortality rate dropped during the following 3 months. It was noted that cement is the least surface where mortality was recorded, where Bendiocarb remains longer in mud, followed by the straw (used in huts and ceilings). In the glass (inert) substrate, Bendiocarb persisted longer than all the other substrates. Straw and mud substrates are less porous than the others; glass (high persistence of Bendiocarb), is not absorbent. The cement (the most porous) mortality rate was < 80% after the 3rd month, as well as the mud surface. The straw resulted in mortality rate < 80% after the 4th month. However, the glass treatment mortality rate was < 80% even in the 5th month.

In conclusion, the concentration of Bendiocarb proved to be building material -dependent. Thus, it is important to know the material used in the house intended for IRS before applying the dose /m². However, the residual life of Bendiocarb was 3 months and, therefore, 2 rounds of spray campaign / yr. are required for the Bendiocarb to be effective a few days before the start of the wet-season (Kharif), and a few days also before winter season, where the adults prefer to spend the day inside houses.

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