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THE USE OF TWO BIOLOGICAL FORMULATIONS OF *BACILLUS THURINGIENSIS* AND *BACILLUS SPHAERICUS* IN THE CONTROL OF MOSQUITO VECTORS OF MALARIA IN DRAINS AND RICE FIELDS

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Abstract

Two biological formulations of the microbial agents *Bacillus thuringiensis* and *Bacillus sphaericus,* known by their trade names Vectobac 12 AS and VectoLex CG (Corn Cob) granules, respectively, were obtained from Valent Biosciences Company (formerly Abbott Laboratories) of North Chicago, USA, and applied to control mosquito breeding sites. The *B. sphaericus* formulation was used in trials against *Culex* and *Anopheles* mosquito larvae in gutters at Labadi, Nungua and the estuary of the Chemu Lagoon, whilst the *B. thuringiensis* formulation was used against *Anopheles* species in irrigated paddy rice fields at Akuse in the Eastern Region of Ghana. *B. sphaericus* was applied at 1.8 gm^2 to achieve a concentration for the gutter treatments. In the rice fields, *B. thuringiensis* was applied at increasing doses of 0.095 g m^{-2}, 0.19 g m^{-2} and 0.38 g m^{-2}. The results showed a 99-100 per cent mortality of all larvae with both *B. sphaericus* and *B. thuringiensis* for 5- 16 days after applying the formulations at selected breeding sites. Trials conducted with these formulated microbial agents and their efficiency in controlling mosquito vectors of malaria and filariasis are discussed.

Résumé

OCRAN H. M. & AKPABEY F. : *Application de deux formules biologiques de* Bacillus thuringiensis *et de* Bacillus sphaericus *dans la lutte contre les vecteurs de moustique du paludisme dans les égouts et les rizières* Dans ces expériences deux formules biologiques des agents microbiens *Bacillus thuringiensis* et *Bacillus sphaericus* connus par leurs noms de marque VectoLex CG (épis de maïs) granules respectivement, étaient obtenus de la Compagnie Valent Biosciences (autrefois Laboratoires d'Abbott) de Chicago du Nord, USA et appliquée pour enrayer les zones de reproduction de moustique. La formule de *Bacillus sphaericus* était appliquée aux essais contre les larves de moustique Anophèle et Culex dans les caniveaux de Labadi, Nungua et dans les estuaires de la Lagune Chemu, alors que la formule de *Bacillus thuringiensis* était appliquée contre les espèces d'anophèle dans les rizières irriguées à Akuse dans la Région de l'Est du Ghana. *Bacillus sphaericus* était appliquée à 1.8 g> m² , pour atteindre une concentration pour les traitements des caniveaux. Dans les rizières *Bacillus thuringiensis* était appliquée aux doses croissantes de 0,095 g> m² , 0,19 g> m² et 0,38 g> m². Les résultats indiquaient une mortalité de 99% à 100% de tous les larves avec *B. sphaericus* et *B. thuringiensis* de 5 à 16 jours après l'application des formules aux zones de reproduction sélectionnées. Ce rapport est essentiellement axé sur les essais menés avec ces agents microbiens formulas et leur efficacité dans la lutte contre les vecteurs de moustique du paludisme et de la filariose.

Introduction

Malaria is one of the most serious diseases in Ghana, killing over 20 per cent of all children aged 5 years and below annually. It also accounts for over 40 per cent of all cases reported at out patients departments in hospitals and clinics across the country (MOH, 1999). It is caused by a parasite, *Plasmodium falciparum,* that is transmitted by the mosquito vector. In Ghana, the major groups of mosquitoes transmitting malaria are *Anopheles gambiae*, *A. funestus, A. melas* and, to a lesser extent, *A. pharoensis* (Gilles & de Meillon, 1968).

Some species of bacteria, especially the bacilli, are microbial agents that are highly potent against mosquitoes (Culicidae) and black flies (Simulidae) that transmit parasitic diseases, such as malaria and river blindness (Charles & de Barjac, 1981). Scientists have applied formulations of these microbial agents in insect control in public health, agriculture and forestry with excellent results. In India, for example, the maintenance department of Bharat Heavy Electricals used an "Environmental Model" to control *A. subpictus*, *A. culicifacies* and *Culex quinquefasciatus*, and several other genera of mosquitoes.

The model included the use of biological agents, Bactoculicide *(Bacillus sphaericus.)* and Sphere *(B. thuringiensis)* in blocked drains and in water accumulated factory in scraps as a supplement to the environmental management with complete success (Dua *et al.*, 1997). VectoLex CG (corn cob granules) and VectoLex WDG (water dispersible granules) have been successfully applied against *C. quinquefasciatus* larvae in highly polluted breeding sites which received waste water from households. Both formulations gave almost complete control in very dense mosquito larval populations from 1 to 4 days (Burges & Hussey, 1971).

The Division of Environmental Biology and Health of the Council for Scientific and Industrial Research (CSIR)-Water Research Institute, in collaboration with the Institut Pasteur in Paris, has isolated over 30 strains of these microbial agents. These strains from Ghana belong to Serotypes H3, H5a, H5b and H6 that are toxic to some insect genera (Singer, 1980; de Barjac *et al.*, 1992). The isolates from Ghana, like most *Bacilli*, are found in soil and soil-aquatic systems (Buchanan & Gibbons, 1974) and are relatively easy to prepare as solutions for laboratory experiments. However, these alkaline preparations cannot be applied in large-scale vector control measures, because they are not stable in storage.

The cost of manufacturing formulations of these pathogens for large scale field applications is prohibitive (Dankwa, personal communication), and this led to the decision to request for biocide samples from Valent Biosciences Company (formerly Abbott Laboratories) of the USA for control against *Anopheles* and *Culex* species locally. The two formulations were applied in field experiments at varying concentrations to test their effectiveness at different mosquito breeding grounds with very good results which are described in this paper.

Experimental

Two broad categories of experiments were conducted in culverts/drains and rice fields. Preparations for the trials involved selection of sites, marking and measuring the area of gutters and fields and sampling of population densities by dipping to ascertain populations of larvae before application of larvicides. Test water analysis of *p*H, turbidity and conductivity of the breeding sites indicated ideal conditions for breeding of *C. quinquefasciatus*, as expected under such filthy conditions. Jittawadee-Rodcharoen *et al*. (1997) reported similar results with *B. sphaericus* against *C. quinquefasciatus* in polluted water in India.

Site preparations and treatment of drains

Three drains/culverts measuring 8.6 m × 0.25 m, 21.8 m × 0.26 m and 31.9 m × 1.2 m were selected based on the presence of larvae. The surface area of each drain was then estimated and used in the estimation of the dosage rates. Thus, 39 g, 10.2 g and 38.3 g were applied to sites 1, 2 and 3, respectively, so that the effective dosage rate of 1.8 g m^{-2} of *B. sphaericus* (Vectolex) formulation was achieved.

The drains selected for treatments were at Nungua, Labadi and Chemu. The Chemu estuary was populated mostly by *C. quinquefasciatus* with a few *Anopheles* species, whilst at Nungua and Labadi there were virtually only *A. gambiae* and *A. funestus* present.

Pre-treatment sampling of larvae was carried out prior to larvicide application. Five samples were collected from each of the three sites using the normal dip method with a small 200 ml ladle.

Application of larvicide was by hand broad-casting. Treatment of the drains or culverts was done at the same concentration of 1.8 g m^{-2} on day 1. The average volume of water for the five dips per site was recorded. Samples of the non-target fauna found at the experimental sites were also collected and identified to be studied in detail in future experiments.

Site preparations and treatment of rice fields

The rice plots measured were each 154 m^2. There were 12 plots of this size; six were used as treatment plots and six as controls at the Akuse rice farms. The whole system was irrigated by gravity from a system of irrigation canals and rice was planted in all the fields in rows. *B. thuringiensis*, the biological larvicide formulated as Vectobac 12 AS by Valent Biosciences Company of North Chicago (formerly Abbott Laboratories) was used. Three different concentrations were selected and treated in duplicate as follows: 0.095 g m^{-2}, 0.19 g m^{-2} and 0.38 g m^{-2}. An average of 20 dips was taken for each plot at specified points using a 200-ml dipper to cover the entire plot.

A hand operated pressurized Hudson X – Pert Sprayer was used in applying the larvicide to the field. The measured volume of stock larvicide was diluted to 10 litre and mixed in the spray tank. The tank was then pressurized to four bars and the larvicide released into the field through calibrated nozzles designed to release the solution in 10 min. to ensure good mixing and coverage in the plots without undue dilution. Post-treatment sampling was carried out after 24 h, followed at selected intervals until 10 days later.

The trials were set up to determine the LC 100 at the selected dose. However, the dose-mortality ratio at the highest concentration of 0.38 g m^{-2}, (0.36 – 0.39) (99% C.I.) always proved to be 100 per cent over a period, usually 2 days, which normally is the result expected in a successful field trial. Determination of mortality was by percentages based on the difference in numbers between live larvae and dead larvae on each day of sampling. With the rice farms, mortality increased with increase in the concentrations applied. An analysis of the data can provide an estimate of the LC_{50} using arithmetic scale. However, in this study only simple computation of percentage mortalities was done.

Results

Post-treatment evaluation

Culverts/Drains. The post treatment samples showed that *B. sphaericus* has a recycling effect on the larvae. Post treatment sampling was carried out on days 2, 3, 6 and 10. A few larvae were found on the second day and no larvae were found until day 10, when the experiment was concluded, confirming the persistence of *B. sphaericus*.

Rice fields. No live larva was found between 24 and 48 h after application of larvicide. Only dead larvae were present, confirming the high potency of the *B. thuringiensis* aqueous formulation. The results of the trials are shown in Tables 1 – 3 and Fig. 1. The numbers of dips were five per site and these were taken at the same spot in each plot as much as possible. The sample water totaled 1 litre per plot for all the 12 plots so that there was no bias in the sampling method with regard to the numbers of larvae collected per dip. The population of larvae increased steadily as the rice seedlings also matured until there were sufficient larvae for treatment to be carried out.

Controls and non-target fauna. The control animals survived throughout the experiments. Reduction in the numbers of larvae and pupae for the controls was, however, observed. This was the result of pupation and adult emergence during the 2 weeks of the experiments. The non-target aquatic insects collected 24 h after treatment in the rice farms were identified by the hydrobiologist. The results, presented in Table 4, could only be interpreted qualitatively as no pre-treatment collection was undertaken in contrast to the experiments in the US by Mulla *et al.* (1984a), where comparative pre- and post

TABLE 1

Treatment with B. sphaericus *(Vectolex CG fomulation) against* C. quinquefasciatus *and* A. funestus *in drains in Labadi*

Site	*Dose (g m^{-2})*	*No larvae (surviving)*					
		Pre treat	*24 h post*	*48 h post*	*72 h post*	*7 days post*	*14 days post*
T1	1.8 g m^{-2}	3328	485	0	0	0	0
C1	-	3211	2965	231	89	287	252
T2	1.8 g m^{-2}	2105	82	0	0	0	0
C2	-	3897	625	238	155	167	184
T3	1.8 g m^{-2}	3611	181	0	0	0	0
C3	-	2138	2324	221	189	163	113

T - Treatment
C - Control

TABLE 2

Results of trials with B. sphaericus *on* C. quinquefasciatus *and* A. funestus *in the Greater Accra Region - Chemu esttuary*

	Pre-treatment measurements				*Pre-treatment*		*Post-treatment*			
No. of site	*Conc.*	*Average vol. of water sampled (ml)*			*1 h pre-treatment*		*No. larvae*	*No. of larvae*		*No. of laevae*
	g m^{-2}	*Day 1*	*Day 7*	*Day 14*	*No. of pupae*	*No. of larvae*	*24-h post*	*48 h*	*3 days*	*7 days*	*2 weeks*
T1	1.8 g m^{-2}	960	900	800	213	8983	485	64	0	0	0
C1	-	990	1000	890	356	5660	5532	231	103	303	452
T2	1.8 g m^{-2}	940	900	1000	255	6008	15	0	0	0	0
C2	-	950	880	960	2131	3224	3545	238	66	83	566
T3	1.8 g m^{-2}	980	900	750	987	8562	98	23	0	0	0
C3	-	900	800	1010	2044	1258	2130	221	956	700	703

T - Treatmentwith larvicide; C - Control

Test conditions: Water temp. 29.5; Conductivity 61 µS; Air temp.32 °C; *p*H 8.1; Turbidity185; DO - BOD 0.204-2.41 29.5°C.

treatment studies of the non-target organisms were carried out.

Discussion

The results obtained from the trials showed an almost 100 per cent mortality at 0.38 g m^{-2} 24 h after treatment of the rice fields and less than 100 per cent with the other two lower concentrations. This shows that *B. thuringiensis* is highly toxic to mosquito larvae as reported by Mulla & Darwazeh (1984b). It was observed that the species composition remained constant, with *A. gambiae* complex being predominant, followed by *A. funestus*, as studies by Dossou-Yovo *et al*. (1995) and Doannio, Dossou-Yovo & Diarrasouba. (2002) have shown. The presence of larvae 7 days

TABLE 3

Treatment of rice farms at Akuse with Vectobac 12 as B. thuringiensis *against* A. gamblae/A. funesrus *larvae*

Site	*Dose (g m^{-2})*	*No. of larvae surviving*					
		Pre-treat	*24 h*	*48 h*	*72 h*	*7 days*	*14 days*
T1	0.095	152	81	26	18	12	17
T2	0.095	178	72	36	28	8	16
T3	0.19	269	58	16	10	5	0
T4	0.19	304	62	9	6	3	0
T5	0.38	360	14	0	0	0	0
T6	0.38	407	10	0	0	0	0
C1	-	254	263	113	72	80	71
C2	-	246	202	152	112	105	95
C3	-	302	211	171	120	107	99

T - Treatment; C - Control

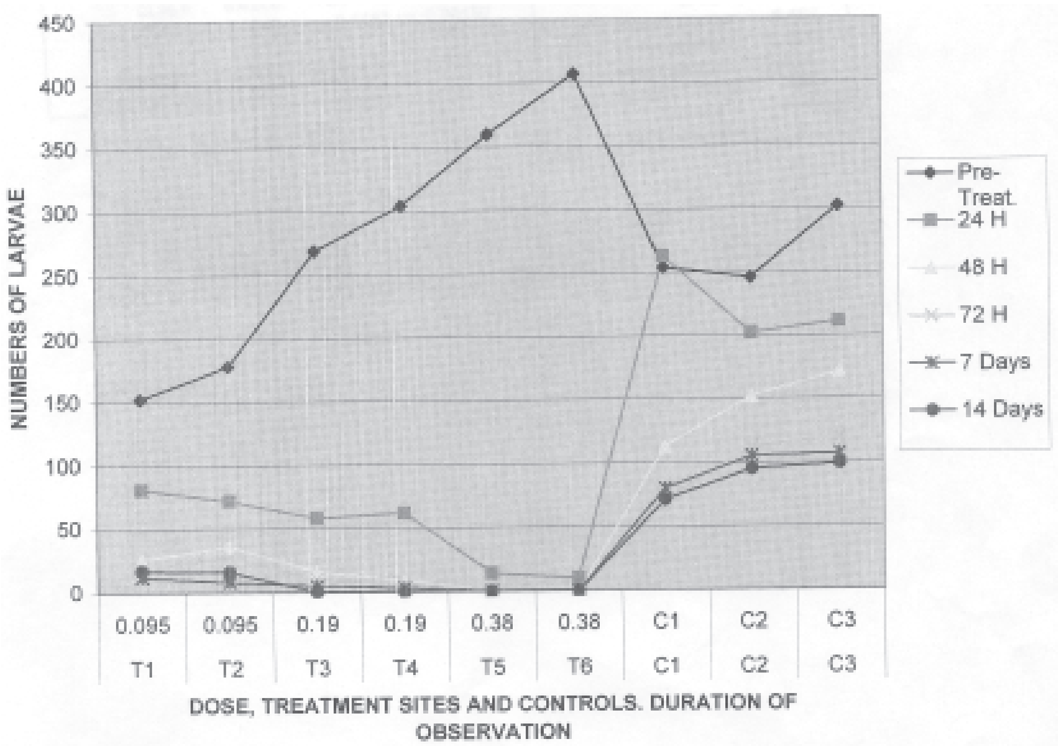

Fig. 1. Treatment of rice farms at Akuse with Vectobac 12 as (b. thuringiensis) against A. gambiae'A. funestus larvae.

2

ANALYSE PROBIT

TEST INFORMATIONS: 29/07/2002 DATE TEST

FILE: T020712

CODE IRU: B9617

INSECTICIDE: B.T. H14

FORMULATION & LOT: VECTOBAC 12AS/ 16-678-N9

26 : Tempera
7.2 : Ph test
60.2 : Conduct
10.1 :Turbidity
28 : Tempera
10 : Contact
250 Discharge

CONTROL:	N	R	PT
SURVIE	100	100	0.00%
MORTALITE	100	2.06666667	2.07%

OBSERVATION:

					LOG	POURCENTAGE		
	SEL	CONC.	N	R	CONC.	R/CORR	%/CORR	PRED.
1		0.38	407	16	-0.420	7.7	1.90%	2.68%
2		0.38	360	14	-0.420	6.7	1.86%	2.68%
3		0.19	304	65	-0.721	60.0	19.72%	16.28%
4		0.19	269	62	-0.721	57.6	21.42%	16.28%
5		0.095	178	72	-1.022	69.8	39.19%	48.58%
6		0.1	152	81	-1.000	79.5	52.30%	45.79%
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								

CL99 =	0.01672	0.03275 << 0.003613		N
CL95 =	0.0278	0.04736 << 0.008347		D. F.
CL50 =	0.0948	0.1191 << 0.06107		CHI-2
				Criteria CHI-2
Slope b =	-3.087	E-Type b =	0.205	Probability
Intercept a =	1.841			Heterogeneïte
				Student t- test
				Factor G
				Nb. iterations

Fig. 2. Analyse probit

after the application of the larvicide at the highest concentration of 0,38 g m^{-2} shows that *B. thuringiensis* does not have a recycling or persistence effect in contrast to *B. sphaericus*.

Malaria transmission studies by human bait catches were not undertaken to ascertain the degree of influence of perennially irrigated rice farms on stable or unstable malaria. This study was carried out mainly to verify the efficacy of bio-larvicides on local mosquito vectors of malaria.

As already shown, these experiments prove that formulated biological larvicides are effective in the control of aquatic stages of mosquitoes. Basically, these trials have proved that the two formulations of biological agents can be successfully applied against breeding sites of mosquitoes with little or no adverse impact on the environment. Both larvicides are effective in clean and polluted water.

It was highly effective against *C. quinquefasciatus* in much polluted drains that received waste water from households. Jittawadee-Rodcharoen *et al.* (1997) reported results of experiments with a formulation of *B. sphaericus* conducted in a district, where breeding

TABLE 4

Evaluation of non-target fauna present - Post - B.t. treatment

Predators of mosquito larvae	*Order*	*Sub-order*	*Family*	*Sub-family*	*Genus*	*Species*
1	Hemiptera	-	Hydrometridae	-	*Hydrometra*	-
2	-	-	Belostomidae	-	*Diplonychus*	-
3	-	-	Gerridae	-	*Limogonus*	-
4	Diptera	Nematocera	Tanypodinae	-	-	-
5	Coleptera	-	Gyrinidae	-	*Cybister* (larva)	
6	Odonata	-	Ceongriidae	-	-	-
			Pseudagrion	-	-	-
7	Odonata	Anisoptera	Gomphidae	-	Too yoiung to identify	
Non-predators						
1	Ephemer-optera	-	Baetidae	-	*Baetis*	-
					Centroptilum	-
2	Coleoptera	-	Elmidae (adult)	-	-	-

Note:
This is a qualitative assessment of the presence of other aquatic insects in this experimental trial of Vectobac 12 AS in a rice field. The numbers of laevae at the time of treatment could have been higher but for the feeding habits of the predators which are known to have a preference for mosquito larvae. This may have had a negative impact on numbers. There were no comparative studies of pre-and post-treatment numbers of organisms as pre-treatment sampling was not carried out.

of *C. quinquefasciatus* was intense, by using aqueous solutions in previously cleaned gutters. The success of control was measured by the reduction in the numbers of aquatic stages. Although *B. sphaericus* was effective for 4 days, the gutter cleaning did not significantly affect the outcome of the results.

The dosage applied in these experiments at Akuse rice farms gave 100 per cent reduction of population for 2 weeks at the concentration of 0.38 g m^{-2}. It was assumed that the 1st and 2nd instar larvae would die at this dose, so only mature stages were used in the evaluation. No obvious adverse effect was observed on the non-target fauna, though this was not measured in quantitative terms as reported by Mulla *et al.* (1984a). The products are also entirely non-toxic to man and all vertebrates generally. Results of work carried out by Koudou *et al*. (2005) shows that malaria vectors infective rates become significantly elevated when associated with irrigated rice agriculture, thus, confirming the idea that irrigated rice farms cause an increase in malaria transmission throughout the year. This is the more reason why cultivation during the dry season by irrigation should be monitored and controlled for mosquito breeding.

Cultivation of paddy rice is practiced on an extensive scale in southern and northern Ghana around human settlements, providing ideal conditions for the breeding of *Anopheles* species. It has been reported by Koudou *et al*. (2005) that the location of human habitation near irrigated rice fields and the practice of double rice cultivation influence the transmission dynamics of malaria in rural areas in central Cote d’ Ivoire. However, recent studies concluded that, for most parts of sub-Saharan Africa that are characterized by stable malaria transmission, the introduction of irrigated agriculture had little or no impact on malaria transmission (Ijumba & Lindsay, 2001; Ijumba, Mosha & Lindsey, 2002; Sissoko, Dicko & Briët, 2004). This may depend on whether the area is an endemic malaria zone, where rainfall and other climatic conditions do influence transmission.

Conclusion

The sample of VectoLex CG, a granular formulation of *B. sphaericus*, obtained from Valent Biosciences Company, is an effective biological control agent against larvae of *A. gambiae* complex and other anopheline species as well as *C. quinquefasciatus* in drains and culverts, and will be useful in an integrated vector control programme under local conditions. Prevention of malaria using formulated biological pathogens that are almost exclusively pathogenic to mosquitoes and black flies offers an additional method of control of malaria.

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