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Full Length Research Paper

An assessment of the use of formulations of the microbial agents *Bacillus thuringiensis* and *Bacillus sphaericus* in the control of larvae of mosquitoes that cause malaria and filarial diseases

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Two biological formulations of microbial agents *Bacillus thuringiensis* and *Bacillus sphaericus* known by their trade names as Vectobac 12 AS and VectoLex CG (Corn Cob) granules, respectively were obtained from Abbott Laboratories of North Chicago, USA. The *B. sphaericus* formulation was used in trials against *Culex* mosquito larvae in choked gutters at Labadi, Ashaiman and Nungua in the Greater Accra Region, whilst the *B. thuringiensis* formulation was used against *Anopheles* sp. in paddy rice fields at Akuse. The results indicate a 99 to 100% mortality of all larvae with both *B. thuringiensis* and *B. sphaericus* for 5 to 16 days after applying the formulations at the breeding sites. Only small quantities of the products were needed to kill hundreds of larvae within 24 to 48 h. The formulations act on filter-feeding and grazing aquatic insect larvae after ingestion. They do not affect non-target aquatic insects and they are entirely harmless to humans. In this report, larvicide control using bio-larvicides has been proposed as one reliable method of reducing disease prevalence by killing the larvae at their breeding sites thus interrupting the life cycle of the mosquito vector at the larval stage.

Key words: Mosquito, larvae, formulations, breeding site.

INTRODUCTION

In Ghana, malaria is the number one cause of morbidity accounting for 40 to 60% of out- patient. It is also the leading cause of mortality in children under five years, a significant cause of adult morbidity and the leading cause of workdays lost due to illness (Ankomah and Asenso-Okyere, 2003). Malaria presents a serious health problem in Ghana. It is hyper endemic in Ghana, with a crude parasite rate ranging from 10 to 70% with *Plasmodium falciparum* dominating. It is the number one cause of morbidity accounting for over 40% of out-patient attendance in public health facilities with annual reported cases of about 2.2 million between 1995 and 2001 (Centre for Health Information Management, Ghana Health Service, 2003).

Malaria is a major killer in Ghana and also the leading cause of mortality among children under five years old (UNDP, 2002). The disease accounts for an average of 13.2% of all mortality cases in Ghana and 22% of all mortalities in children under 5 years. In the case of pregnant women, out of the total number reporting at the health institutions, 13.8% suffer from malaria and 9.4% of all deaths in pregnant women (Antwi and Marfo, 1998) . It is estimated that malaria prevalence (notified cases) is 15,344 per 100,000 with a malaria death rate for all ages being 70 per 100,000. In the case of the 0 to 4 years, it is 448 per 100,000 reported for the year 2000 (United Nations, 2003) . The disease is also the leading cause of workdays lost due to illness in Ghana and thereby contributing more to potential income loss than any other disease. According to Asenso- Okyere and Dzator (1997), average 3 work days is lost per fever episode by the patient and 2 work days by the caretaker. The value of these days lost to the management and treatment of fever per episode is US$ 6.87 and this formed about 79% of the cost of seeking treatment in 1994. In another study

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by WHO (1992), malaria accounted for 3.6 ill days in a month, 1.3 work days absence and 6.4% of potential income loss in Ghana for 1988/89. The disease again is responsible for 10.2% of all healthy life lost as a result of diseases, making it the chief cause of lost days of healthy life in Ghana (Ghana Health Assessment Team, 1981).

Several species of the anopheles mosquito carry the four species parasites namely, *P. falciparum, Plasmodium vivax, Plasmodium ovale* and *Plasmodium malariae*, which cause malaria in humans. Epidemiological analysis in Ghana has revealed that only three species of the *Plasmodium* are present; *P. falciparum* (80 to 90%), *P. malariae* (20 to 36%) and *P.* ovale (0.15%). The *P. falciparum* is thus the predominant parasite species carried by a combination of vectors. The principal vectors are the *Anopheles gambiae complex*, which is the most widespread and it is difficult to control, and *the Anopheles funestus* accounting for 95% of all catches (MOH, 1991).

The coastal zone falls into two eco-epidemiological areas. Just along the coast is the coastal lagoons and mangrove swamps. The principal vector is the *Anopheles melas,* which breeds in the lagoons and swamps. The zone also lies in the Coastal Savanna, which stretches from the lower Volta Region through the Accra Plains to the lower Central Region. Malaria transmission is intense and perennial, but markedly reduced during the dry season especially in the coastal savanna.

The location and severity of malaria are mostly determined by climate and ecology (Gallup and Sachs, 2001). The area of potential transmission is controlled by climatic factors such as temperature, humidity and rainfall as well as the socio-economic conditions of the population. These factors influence the development of both the vector and the parasite.

Cultivation of paddy rice is practiced on an extensive scale in southern and northern Ghana, providing ideal conditions for the breeding of *Anopheles* and *Culex* mosquitoes. The fields are kept waterlogged by irrigation for long periods of time as the rice grows, therefore providing good breeding grounds for the aquatic stages of the mosquito. *Anopheles* is a medically important genus of mosquitoes, which transmits human malaria throughout the year; it requires clean oxygenated water to breed. Breeding sites may include stored- water containers in house holds, fresh water ponds and along the edges of lakes.

Much effort has been put into reducing the prevalence of the disease, but no significant impacts have been made due to lack of funding, poor commitment by all stakeholders and poor implementation of control programmes. Ideally, it is suggested that malaria control should be approached in a holistic manner involving all aspects of control of the disease and involving all stakeholders, especially the communities.

Experiments carried out in US and India demonstrated that certain bacteria were very potent microbial agents that were highly effective against certain dipterans such as mosquitoes and simullids that transmit parasitic diseases such, as malaria and river blindness (Charles and de Barjac, 1981). Scientists have applied 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 has used an “Environmental Model” to control *Anopheles subpictus*, *Anopheles culicifacies, Culex quinquefasciatus* and several other genera of mosquitoes. The model included the use of biological agents, Bactoculicide (*Bacillus sphaericus*) and Sphere (*Bacillus thuringiensis*) in blocked drains and in water accumulated factory 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. Both formulations gave almost complete control in very dense mosquito larval populations from 1 to 4 days (Burges and Hussey, 1971).

The Environmental Biology and Health Division of the Water Research Institute (WRI) of the Council for Scientific and Industrial Research (CSIR), has isolated over 30 strains, of which at least two are as active as the Nigerian strain 2362 currently used in commercial formulations in Laboratories in the US. These strains from Ghana belong to Serotypes H3, H5a and 5b and H6 in which all are almost toxic to some insect genera, (Singer, 1980; de Barjac et al., 1992). These isolates from Ghana are located in soil and soil-aquatic systems, (Buchanan and Gibbons, 1974) and it is relatively easy to prepare effective laboratory volumes as alkaline solutions. However, since large-scale laboratory formulations cannot be easily manufactured, the preparations cannot be applied using large-scale vector control measures even though they are effective in the laboratory. The cost of formulation is prohibitive (Dankwa, pers. comm.), and this led to the decision to request for samples from Valent BioSciences Company (formerly Abbott Laboratories) of the US for control against *Anopheles* and *Culex* species locally. Valent BioSciences Co. supplied samples of Vectobac 12 AS; a formulation of aqueous suspension of *B. thuringiensis;* and VectoLex CG, granular formulation of *B. sphaericus*. The two formulations have been applied in field experiments involving application at selected concentrations at different mosquito breeding grounds with very good results. Preparations for the trials involved selection of sites, marking and sampling of population densities by dipping to ascertain the populations of larvae before the application of larvicides. Test water analysis of pH, turbidity and conductivity of the breeding sites, indicated ideal conditions for the breeding of *C. quinquefasciatus* as expected under such polluted conditions. Jittawadee-Rodcharoen et al. (1997), reported similar results with *B. sphaericus* against *C.*

quinquefasciatus in polluted water. The results indicated that *B. sphaericus* does have a recycling effect since populations of larvae remained completely suppressed during the 2 to 3 weeks of post-treatment observation.

MATERIALS AND METHODS

Study sites

Sites for the trials were selected at Ashaiman (2 sites), a peri-urban slum area near Tema, Nungua (1), and Labadi (1) in Accra for the drains and Kpong Farms at Akuse for the Rice Fields. All the sites are located in Ghana, West Africa. These sites were selected based on the fact that slum dwellings have been noted to be the breeding grounds for mosquitoes since there is no planned infrastructure and non-existence of drainage systems. Also the rice fields were selected considering the results from work carried out by Koudou et al. (2005), who 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.

Pre-treatment evaluation

Each treatment site had its corresponding untreated site as a control. The product used was Vectolex CG, (*B. sphaericus*) Batch No. 32-094-BR, a granular formulation.

Treatment of drains

The area for each portion of drain was measured with a tape measure and calculated in square meters for the weight of product to be applied, per unit area. Pre-treatment sampling of larvae was carried out prior to larvicide application. Five samples each of all eight sites were collected using the Normal Dip method with large ladles.

Application of granules of Vectolex was by hand broadcasting. Treatment of these drains or sites was done at the same concentration of 1.8 g/m^2 on day 1. The average volume of water for the 5 dips per site was recorded. Samples of the non-target fauna found in the experimental sites were also collected. Records of experimental conditions, such as pH, turbidity, conductivity of the site, water temperature and oxygen content were taken. These parameters are necessary for the correct interpretation of data.

Post-treatment evaluation

Post-treatment evaluation was carried out on the 2nd, 3rd , 4th and 14th days, making a total of two weeks of surveillance after the application of larvicide. Five dips were taken at the same spots as was done before treatment to make the results comparable. An unexpected heavy downpour of rain brought an abrupt end to the experiments on the15th day since the information collected after the rain could not be exploited.

Treatment of rice fields

Each 154 m^2 plot was irrigated by gravity and rice was planted in all the fields in rows. *B. thuringiensis*, the biological larvicide formulated as Vectobac 12 AS by Valent BioSciences was used. Three different concentrations were prepared and used in the treatment in duplicate as follows: 0.095, 0.19 and 0.38 ml/m^2. Six similar plots were used as controls, two for each concentration.

Sampling of the plots was done with dippers, with an average of 20 dips taken for each plot at specified points to cover the entire field. A hand-held pressurized Hudson X – Pert Sprayer was used in applying the larvicide that was diluted in 10 L of water and the pressure pumped to 4 bars. Spraying was done in a forward and backward movement to ensure complete coverage of each treated field. Post-treatment sampling was done 24 h, 7 days and 12 days later.

RESULTS

Post-treatment evaluation

Only 3rd and 4th instar larvae were used in assessing mortality and interpreting the data in general, since they are bigger and more easily identified. 1st and 2nd instar larvae are known to be more susceptible to poisoning (Mulla et al., 1984) and therefore not taken into consideration (Tables 1 and 2).

DISCUSSION

Between 24 and 48 h after the application of larvicide in the rice fields, no larva was found alive confirming the high potency of the *B. thuringiensis* aqueous formulation (Table 1).

The results were similar for drains treated with *B. sphaericus.* Within 48 h, almost all larvae were dead. The product does not, however, affect pupae since they do not feed. All data were recorded for pre- and post-treatment in tables. The volumes and the number of larvae collected are also presented in the tables. The sampling recovered many dead larvae 24 h after treatment. The very few moribund larvae were all found to be dead after one week, leaving only their skins in the water. Figures 1 to 5, show the graphical representations of the results of the trials.

To obtain statistically comparable numbers of larvae collected in an average of 5 dips, the numbers of larvae were based on a volume of 1.0 L of water collected, which in effect, averaged out all larvae sampled per site.

A heavy rainfall prevented the follow-up of the trials after 2 weeks due to flooding of the experimental sites, including the controls. Data could not be exploited after this rain, which ignited water flow in all gutters.

The non-target aquatic insects collected 24 h after treatment were identified by the hydro-biologist. The results, presented in Table 1, could only be interpreted qualitatively as collection prior to treatment was not carried out in contrast to the experiments in the US by Mulla and Darwazeh (1984), where comparative pre- and post-treatment studies were carried out. This activity has been planned for the next series of trials.

The results obtained from these trials were good, with almost 100% mortality at 0.38 ml/m^2 24 h after treatment and shows that *B. thuringiensis* is highly toxic to mosquito larvae, as reported by Mulla and Darwazeh (1984). The

Table 1. Effects of *B. thuringiensis* on mosquito larvae in rice fields at Akuse.

No. of site	Pre-treatment measurement				Pre-treatment		Post-treatment				
	Conc. (g/m²)	Average vol. water sampled (ml)			1 h pre-treatment		No. of larvae	No. of larvae	No. of larvae	No. of larvae	No. of larvae
		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	650	755	870	418	582	18	0	0	0	0
C1	-	900	880	940	321	533	161	154	77	69	231
T2	1.8	800	840	700	654	418	21	0	0	0	0
C2	-	900	760	740	741	241	500	221	45	305	298
T3	1.8	800	760	840	625	850	12	0	0	0	0
C3	-	720	880	700	401	421	1008	109	23	268	352

T = Treatment with larvicide; C = Control. Test conditions: Water temperature = 29.5°C; Air temperature = 31.5°C; Conductivity = 98 uS; Turbidity = 102 NTU; pH = 7.9; DO = - ; BOD = 0.011 - 0.19.

Table 2. Evaluation of non-target fauna: 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 young to identify	
Non-predators						
1	Ephemer- optera	-	Baetidae	-	*Baetis centroptilum*	-
2	Coleoptera	-	Elmidae (Adult)	-	-	-

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 larvae 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. Efforts will be made to assess the impact of predators quantitatively in future trials.

presence of larvae 7 days post-treatment shows that *B. thuringiensis* does not have a recycling effect as does *B. sphaericus*.

The results of this study indicated that formulated biological larvicides are effective in the control of aquatic stages of mosquitoes. Basically, the trials showed that the two formulations of biological agents can be successfully applied against breeding sites of mosquitoes with little or no

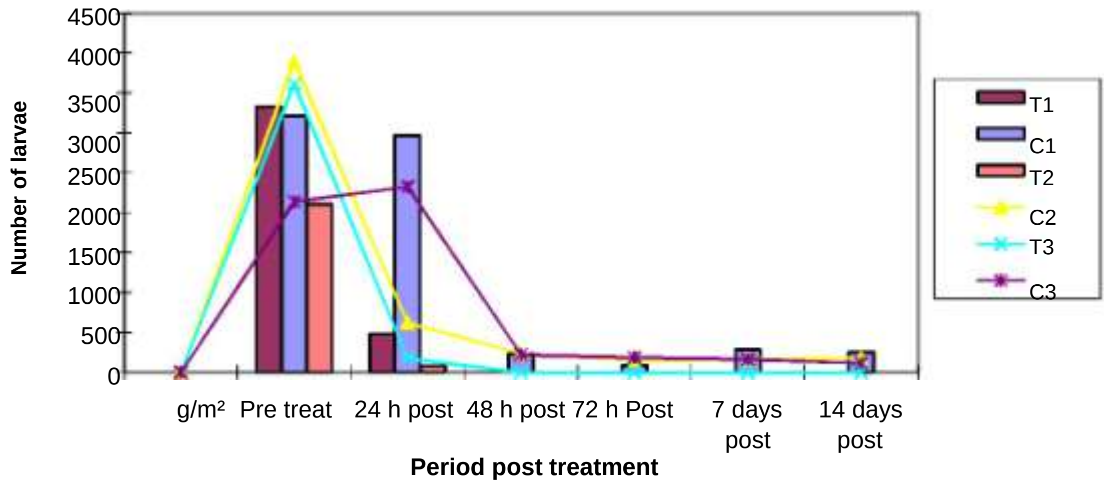

Figure 1. *B. sphaericus* treatment against *C. quinquefasciatus* and *An. funestus* in drains in labadi at concentration of 1.8 g/m^2.

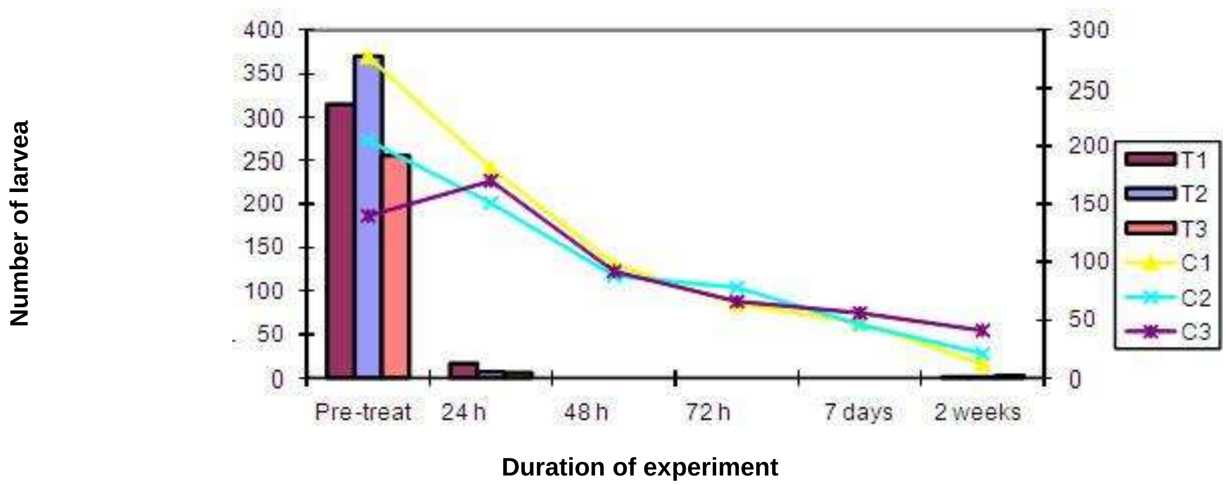

Figure 2. Mortality distribution of larvae of *An. gambiae* and *C. quinquefasciatus* treated with *B. sphaericus* in Nungua drains.

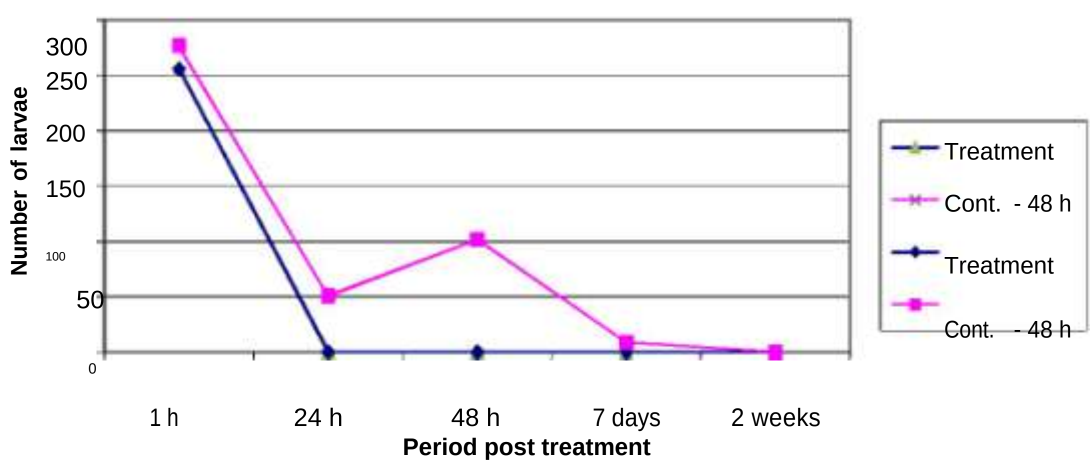

Figure 3. Mortality distribution of *C. quinquefasciatus* treated at 1.8 g/m^2 in gutter at Ashaiman-Valco flats.

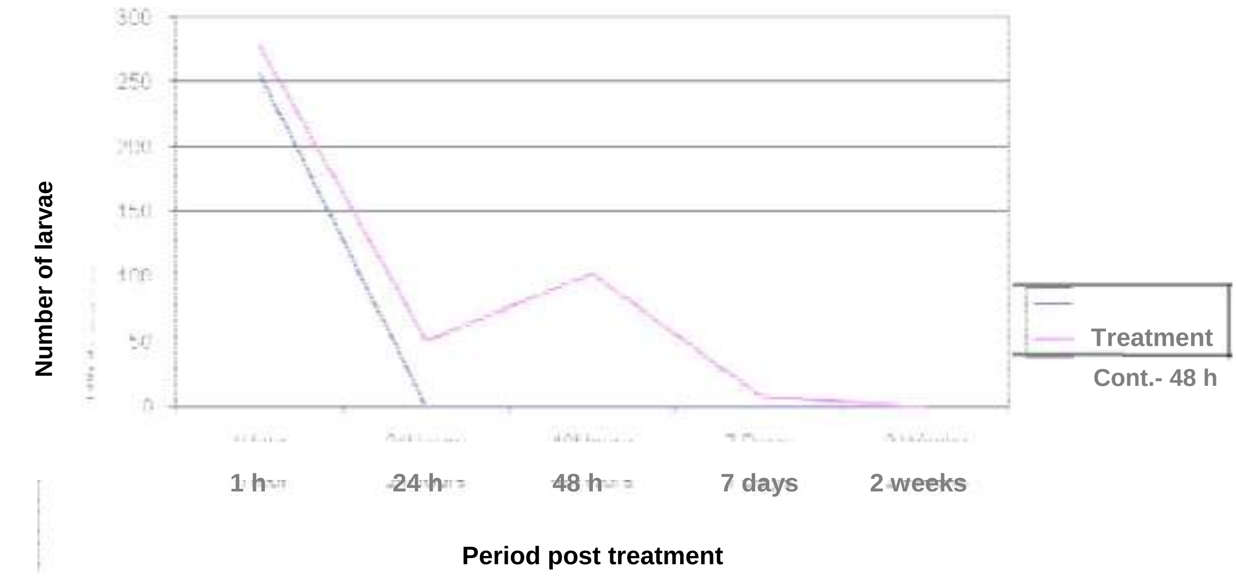

Figure 4. Mortality distribution of *C. quinquefasciatus* in a gutter at Ashaiman (Top Bay Plaza).

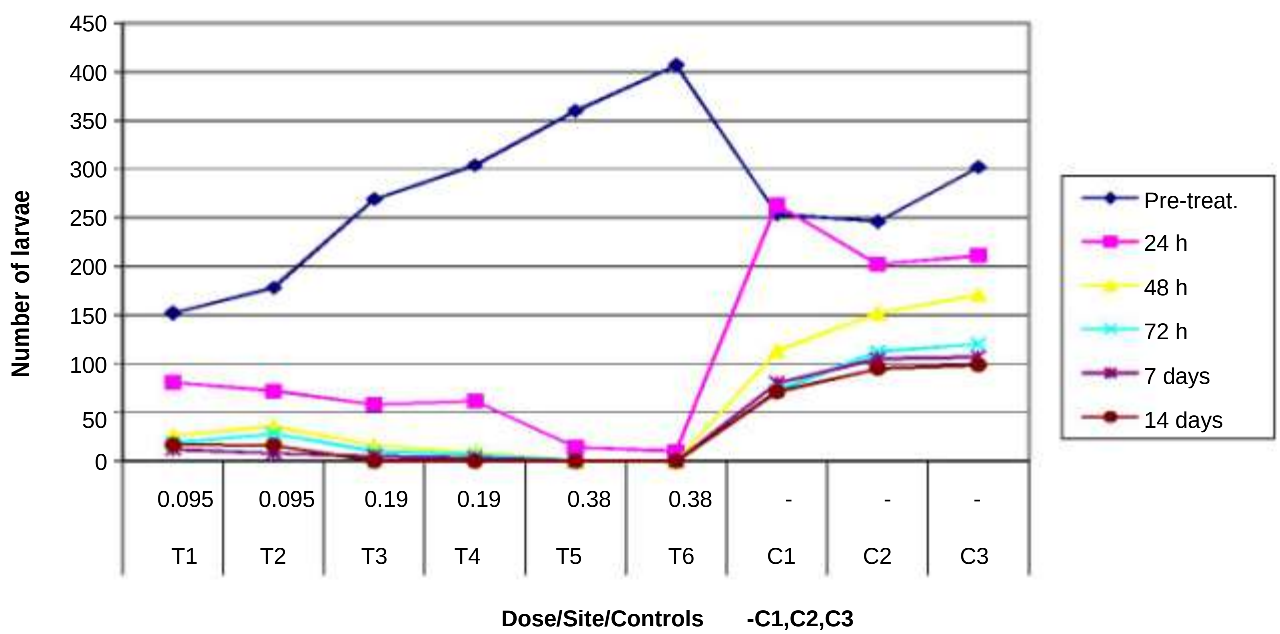

Figure 5. Akuse rice farms – Treatment of rice plots and their controls

adverse impact on the environment. According to Wilson et al. (2005), *B. thuringiensis* serotype H-14 Berliner (*B.t.*) can be used as a biological control agent against aquatic dipteran pests such as blackflies and mosquitoes. It is highly selective and is safer for non-target organisms than conventional insecticides. Safety studies have so far shown no harmful effects on bees, vertebrates including man, and most beneficial insects are unharmed even at enormous doses (de Barjac, 1978; WHO, 1979).

The *B. sphaericus* was highly effective against *C. quinquefasciastus* in the polluted drains that received wastewater from households. Jittawadee-Rodcharoen (1984), reported results of experiments with a formulation of *B. sphaericus* conducted in a district where breeding 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 gave 100% reduction of population for 2 weeks. It was assumed that the 1st and 2nd instar larvae would die at this dose. *B. sphaericus* is an effective biological control agent against the larvae of *An. gambiae* and *C. quinquefasciatus* and will be useful in an integrated vector control programme under local conditions. *B. thuringiensis* also demonstrated its high efficacy against the larvae of all three species of mosquitoes treated and it was noteworthy that the *Anopheles* species, which transmit malaria, succumbed totally to this bacterial pathogenic formulation. The data show that in all treated gutters 100% mortality was obtained at all sites except for the Valco flats gutter, where a few survivors were observed after 48 h.

The results were very spectacular with regard to treatments in the rice fields with *B. thuringiensis*. Within 24 h over 99% to 100% of all the larvae were dead.

The control larvae survived throughout the experiments, however, there were reductions in their numbers and in those of the pupae. This was probably the result of some larvae maturing to pupae and some adult emergence of the pupae during the two weeks of the experiments.

No obvious adverse effect was observed on the non-target fauna, even though this was not measured in quantitative terms. The products are also entirely non-toxic to humans and all vertebrates generally (Table 2).

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