International Journal of Ecology and Ecosolution
Vol. 3(3), pp. 30-39, July 2016
ISSN: 2437 - 1327
Full Length Research Paper

Invertebrate communities improve the efficiency of the biological pesticide *Bacillus thuringiensis* var. *israelensis* (Bti) in mosquito control: A case study in Cameroon, Central Africa

Alvine Larissa MEYABEME ELONO[1]*, Matthias LIESS[2] and Sabine DUQUESNE[3]

[1]Department of Forestry, University of Dschang, PO.Box. 222 Dschang, Cameroon.
[2]UFZ – Helmholtz Centre for Environmental Research, Department of Conservation Biology, Permoserstrasse 15, D-04318 Leipzig, Germany.
[3]UBA, Federal Environment Agency, Section Plant Protection Products, Wörlitzer Platz 1, D-06844 Dessau-Rosslau, Germany.

Accepted 15 July, 2016

ABSTRACT

We investigated the influence of different combinations of natural aquatic invertebrate communities and the biological insecticide *Bacillus thuringiensis* var *israelensis* (Bti) on the establishment of larval populations of mosquitoes in temporary ponds in the field. The study involved two experiments so called “Bti and community” and “community density” experiments respectively. In the “Bti and community” part, three treatments were tested: added aquatic invertebrate community alone (“com”), Bti alone (“Bti”), and a combination of added invertebrate community and Bti (“comBti”). The “community density” experiment included three setups, all treated with Bti but having different densities of aquatic invertebrate communities (that is, “low”, “medium” and “high”). The two species of mosquitoes that established in the ponds were *Anopheles* spp. and *Culex* spp. In both experiments and after 24 h following the application of Bti, larval abundance of these mosquitoes decreased by about 78 to 99% in the setups where the biological pesticide was used. However, the recovery of mosquito larvae in these ponds was fast, and the most important in low community density conditions (“Bti” and “low”), moderate in medium community density (“medium”) and did not occur in high community density (“high” and “comBti”). By contrast with the combined treatment of invertebrate community and Bti (“comBti”), the abundance of mosquito larvae in the setup treated with invertebrate community alone (“com”) showed an increase during some days and then a progressive decrease until the end of the experiment. These results suggest that the treatment with Bti alone was efficient but only for a short time as recovery occurred; the addition of community alone was efficient in a longer term whereas the combination of Bti and added community was efficient in both the short and longer terms. This implies that the efficacy of larval control of mosquitoes by Bti was improved with high community density.

Keywords: Mosquito control, biological pesticide, *Anopheles* spp., *Culex* spp., community, temporary ponds.

*Corresponding author. E-mail: meyabemealvine@yahoo.fr. Tel: +237 679 28 96 94. Fax: +237 233 45 13 81.

INTRODUCTION

Since the discovery of mosquitoes as vectors of diseases (Ross, 1897), several control strategies have been developed and implemented (Ramirez et al., 2009). In the 1980s, chemical insecticides were largely used in mosquito control. However, the resistance by the target populations to these insecticides (Hemingway and

Ranson, 2000) and the appearance of unwanted damages to the environment (Becker et al., 2003) led to the exploration of alternative methods such as biological control. Biological control implies the use of beneficial organisms (e.g. bacteria, parasites, predators, competitors) for reducing pests or mitigating pest effects.

Larvicides based on the bacterium *Bacillus thuringiensis* var. *israelensis* (Bti) are considered to be among the safest pesticides in pest control programs (Boisvert, 2005). Bti has a great success in mosquito control in many countries worldwide (Becker et al., 2003; Lacey, 2007). Mosquito larvae are rapidly affected and eliminated within 24 h following ingestion of Bti spores (Amalraj et al., 2000; Aldemir, 2007; Kahindi et al., 2008). However, certain limitations are associated to the use of Bti. For example, it must be ingested to act and thus it is not efficient to pupae and late fourth instars larvae which are stages that do not feed. Also, it becomes quickly unavailable to the filter feeders mosquito larvae due to the fast adsorption of spores to the sediments (Boisvert, 2005); as a consequence, repeated applications are necessary to prevent the resurgence of the next generations of mosquitoes in treated biotas (Hougard and Back, 1992; Becker et al., 2003). Besides, the susceptibility of some non target organisms to Bti (e.g. Chironomidae, Lepidoptera, *Chlorella* sp., *Closterium* sp.) has been reported (Boisvert and Boisvert, 2000). However, natural enemies of mosquito larvae such as Cladocera, Cyclopoida, Notonectidae and Dytiscidae (Knight et al., 2004; Kumar and Hwang, 2006; Marten and Reid, 2007; Meyabeme Elono et al., 2010; Duquesne et al., 2011; Kroeger et al., 2013) are not affected by Bti at recommended dosages for operational treatments (Boisvert and Boisvert, 2000).

In African countries, many mosquito species (e.g. *Anopheles*, *Culex*) are involved in the transmission of dangerous diseases like malaria and lymphatic filariasis (Fontenille and Carnevale, 2006; Camargo, 2008). During the first rainfalls in tropical regions, temporary waters are spontaneously generated in the ground depressions and are used as breeding sites by mosquitoes (e.g. *Anopheles* species) (Chaves and Koeenradt, 2010). This explains the strong positive correlation that is observed between rainfall and abundances of mosquitoes in countries such as Cameroon (Barbazan et al., 1997; Bigoga et al., 2007; Atangana et al., 2009), Ghana (Tuno et al., 2010), Nigeria (Oyewole et al., 2007), and Senegal (Faye et al., 1997).

Moreover, human activities (e.g. sand pits, agricultural irrigation) contribute for amplifying environmental conditions favourable to the breeding of mosquitoes (Mutuku et al., 2006; Simard et al., 2009).

Liess and Duquesne (2014) have proposed a method for pest control in aquatic ecosystems, consisting of the simultaneous use of a two-step method: the first step is the elimination of pest larvae using a biological insecticide targeted specifically against mosquito larvae (e.g. Bti) and prevention of the recolonisation using antagonists such as competing and predatory taxa of the pest larvae. In the present work, we implement this proposed mosquito control method to test its efficiency in the field. The aim is to assess if the introduction of aquatic invertebrate communities can sustain a Bti-based treatment for the larval control of mosquitoes in human-made breeding sites. We conducted two experiments (i) to compare the efficacy of the treatments by Bti alone, community alone, and both Bti and community in combination ("Bti and community" experiment); and (ii) to assess the influence of the density of the community in the recolonisation process of mosquito larvae in ponds after Bti treatment ("community density" experiment).

The study took place during the small rainy season in the wetland of the stream Mbomo that waters the periurban area of Mfou, close to Yaounde, Cameroon (Central Africa). The hypothesis was that the abundance of mosquito larvae after Bti application will be much lower in treatments combining Bti and high community density than in treatments with either Bti alone or high community density alone. The results derived from the present study shall be useful for control strategies of mosquitoes based on Bti in peridomestic temporary ponds during rainy seasons.

MATERIALS AND METHODS

Study site

The investigation was conducted in Cameroon (Central Africa) during the small rainy season from April to June. Study sites were located in the wetland of the stream Mbomo which waters Mfou (3°58'N 11°56'E), a peri-urban area close to the capital Yaoundé. The climate in this region is characterized by 2 rainy seasons (March to June and August to November). The neighbouring residents use the wetland of the stream Mbomo for domestic agriculture of green vegetables among other activities (e.g., digging of sand). At the end of the rainy season when the level of water drops down, small semi natural ponds are created for watering plants. These ponds generally dry out in the course of the dry season. But following the first rainfalls of the next rainy season, they refill.

Experimental design

We selected 16 ponds that are used by neighbouring residents for watering their vegetables. The water was emptied and the top soft layer of the sediments was removed from all selected ponds so that initial conditions, in terms of quantity and quality of the community, are similar at the start of the experiment. Ponds were filled up again with rainfall and infiltrations within 24 h and were left to be naturally colonised by local mosquitoes.

Ponds were then treated with *Bacillus thuringiensis* var *israelensis* (Bti) and/or with introduced invertebrate communities. The formulation of Bti used here was VectoBac. It was obtained from the German Mosquito Control Association – KABS (Waldsee, Germany). The rate of 4 g/m^3 of Bti was applied on the surface of ponds using an 8-L graduated and transparent air pressure sprayer SM-8A (Zhejiang, China). The local invertebrate communities from the surrounding ponds were collected and introduced in the community ponds. This was realised by filtration of 100 L of water to 1 L using a 55-µm mesh. This operation was repeated several times and the 1-litre subsamples were pooled together into a single sample. This single sample of invertebrate concentrate was distributed in appropriate ponds of the two experiments (the resulting mean abundances of invertebrates in treated ponds for each experiment are detailed below). Taxonomic groups collected encompassed Ciliata, Rotifera, Ostracoda, Cladocera (that is, *Ceriodaphnia* spp. and *Chydorus* spp.), Hydracarina, Collembola, Cyclopoida, *Hydra* sp. and insects larvae (that is, Chironomidae, Ephemeroptera, Hydrophilidae, and Odonata). Many of these taxonomic groups are competitors (e.g., Ostracoda, Cladocera, Chironomidae) and predators (Cyclopoida, Odonata) of mosquito larvae. The identification keys which were used were those of Ward and Whipple (1959), Durand and Lévêque (1980), Becker et al. (2003), and Tachet et al. (2003).

"Bti and community" experiment

This experiment included three different setups, i.e. the biological pesticide Bti alone ("Bti", n = 5), added community alone ("com", n = 5), and added community + Bti ("comBti", n = 5). Each pond of the setups "com" and "comBti", received three times 500 ml of the aquatic invertebrate concentrate (obtained as described above) per m^3 of water. No organism was added in "Bti" ponds. Bti and added community were applied once after four days following the start of the experimental work (emptying of the ponds). Aquatic invertebrate communities were introduced in appropriate ponds three hours before Bti. And just before the application of Bti treatment, the mean numbers of invertebrate taxa in the setups "Bti", "com", and "comBti" were 79 ± 20, 167 ± 15, and 167 ± 21 individuals/L, respectively. This experiment lasted around two weeks in total.

"Community density" experiment

This experiment included three setups with Bti treatment and different community densities, that is, "low" (n = 8), "medium" (n = 4), and "high" (n = 4) density. The volumes of 500 ml of the invertebrate concentrate were introduced once in the "low" community ponds, two times in "medium", and four times in "high" community density. In this experiment, aquatic invertebrate communities were introduced in all ponds four days before the treatment with Bti to allow the establishment of the invertebrate populations. On the day of Bti treatment, the mean numbers of invertebrate organisms for the setups "low", "medium", and "high" community density were 72 ± 18, 88 ± 20, 170 ± 30 individuals/L, respectively. The "community density" experiment lasted around three weeks in total.

Monitoring of mosquito larvae and associated invertebrates communities

In the two experiments, the abundances of mosquito larvae were recorded once before Bti treatment and at least two times per week afterwards. Mosquito larvae were sampled by dipping four times a volume of 250 ml at the edge of each pond (adapted standard technique, WHO 1975). The results of the four samples were averaged and the abundance was expressed in number of larvae per dip. Counted larvae were immediately returned to the pond.

The abundances of invertebrate taxa associated to mosquito larvae were recorded once before Bti treatment and at least three times in the post treatment period. Specifically, we took 10 samples of 250 ml from different sides and depths of each pond. The subsamples were pooled together into a single sample and gently stirred. One litre of this pooled sample was filtered through a 55-µm mesh and conserved in a 15-ml brown flask with a mixture ethanol : distilled water (70:30). The remaining part of the pooled sample was returned back to the original pond.

Physicochemical parameters

The physicochemical parameters were assessed once before Bti treatment and twice in the post exposure period in the two experiments. Water temperature, pH, and total dissolved solids (TDS) were measured with an electronic multimeter ExStik EC500 (Walthman, USA); and dissolved oxygen (DO) with an electronic oxymeter ExStik DO600 (Walthman, USA). The variables required for calculating the water surface area and the volume (that is, diameter and depth) were evaluated using a graduated ruler. For the measurements of Chlorophyll *a*, samples of 250 ml were filtered through a 0.45 µm mesh-size Whatmann GF/C glass fiber filter. This filter was kept in acetone during 24 h in the dark and at 4°C for extraction. The optical density of the extract was recorded with a spectrophotometer (DR 2000, Loveland, USA) and converted to chlorophyll a concentrations using the equations of SCOR/UNESCO (1966).

We found no significant differences between setups about physicochemical parameters, neither in the "Bti and community" experiment nor in the "community density" experiment (ANOVA on average and as well as for each time point, $P > 0.05$). Indications of the mean values of the measurements realized just before Bti treatment are given in Table 1.

Data analysis

Data were analysed following three time periods, defined similarly in both experiments. The first time period is the pre-treatment period that describes an overview about the abundances and the distribution of mosquito larvae in the different setups before the application of Bti. The second one is the 24-h post exposure period that shows the impact of Bti on larval abundances of mosquitoes within one day following the application of Bti (short-term period). The third period lasts from the 24-h post-exposure to the end of the experiment (long-term period); this shows if larval populations of mosquitoes recolonise the ponds.

All data were subjected to log (x+1) transformation prior to analyses. One-way ANOVA was used to test for the difference in the mean abundances of mosquito larvae between setups for each period (that is, short-term, 24-h post-exposure, and long-term periods). The factor "treatment type" was used to compare the setups "com", "Bti", and "comBti" in the "Bti and community" experiment. The factor "community density" was used to compare the setups "low", "medium", and "high" in the "community density" experiment. A significant ANOVA was followed by a *Bonferroni* post hoc test for paired wise comparisons between the different setups of the "Bti and community" experiment, and by a *Dunnett-T* post hoc test in the "community density" experiment to compare the setup "low" with the setups "medium" and "high".

The Redundancy Analysis (RDA) was carried out on data collected during the recolonisation period to highlight the influence of the factors "community density" on the community structure of different setups. RDA is a linear constrained multivariate ordination which is appropriate to describe correlations between response variables and predictors in a complex system characterized by many species (Leps and Smilauer, 2003). For this analysis, the factors "community density" was used as predictor, and mosquito larvae and their associated invertebrate taxa were used as response variables. When a significant correlation was detected, the percentage of explained variations by the first ordination axis is given in brackets on the ordination graph. The percentages of explained variations were obtained by multiplying the fit of each species (provided in the results file of RDA) into the ordination space by 100.0 (ter Braak and Smilauer, 2002).

Table 1. Means ± SE of physicochemical parameters in the pre-treatment period of “Bti and community” (n = 15) and “community density” (n = 16) experiments.

Parameters	Experiment	
	Bti and community	Community density
TDS (mg/L)	79 ± 6	120 ± 10
pH	7.4 ± 0.2	7.1 ± 0.2
O2 (mg/L)	5.3 ± 0.6	4.6 ± 0.5
Temperature (°C)	29.6 ± 0.7	28.7 ± 0.4
Chl a (mg/m^3)	64 ± 9	125 ± 17
Suface area (m^2)	0.63 ± 0.1	0.67 ± 0.1
Volume (L)	1053 ± 172	1028 ± 181

The Hotelling’s T^2 test was carried out to check for the difference in the overall community associated to mosquito larvae between Bti- and non Bti- treated ponds (“comBti” and “com”, respectively) in the “Bti and community” experiment. Hotelling’s T^2 test is a multivariate test which is appropriate for comparing multiple variables between two groups (Gotelli and Ellison, 2004). In this test, the overall community is quantified as the means of all invertebrate taxa associated to mosquito larvae cited in the section “Experimental design” above.

RESULTS

The two mosquitoes identified in the present investigation were *Anopheles* and *Culex* species.

“Bti and Community” experiment

“Pre-treatment” period (before Bti treatment)

During the pre-treatment period, no difference was observed in larval abundances of *Anopheles* spp. (“a” in Figure 1A) and *Culex* spp. (“a” in Figure 1B) between all setups including added community alone (“com”), Bti alone (“Bti”), and “ComBti” (Bti + added community) (ANOVA, P > 0.05).

“Short-term” period (24 hours following Bti treatment)

After 24 h following the treatment with Bti, there was a fast decrease of larval abundances of both *Anopheles* spp. (“b” in Fig. 1A) and *Culex* spp. (“b” in Fig. 1B) in the setups “Bti” and “ComBti”. In fact in these two Bti-treated setups and on one hand, larval abundances of *Anopheles* spp. were reduced to 78 and 91%, respectively, compared to the initial abundances. And on the other hand, larval abundances of *Culex* spp. were reduced to 94 and 99% in “Bti” and “comBti” setups, respectively. By contrast in the setup treated with added community alone (“com”), there was an increase in larval abundances of *Anopheles* spp. (Figure 1A) and *Culex* spp. (Figure 1B). During this “short-term” period, the larval abundances were significantly higher in the “com” setup than in the “Bti” and “ComBti” setups for the two mosquitoes *Anopheles* spp. (“b” in Figure 1A, ANOVA: P = 0.034) and *Culex* spp. (“b” in Figure 1B, ANOVA: P = 0.002).

These results show that the larval abundance of mosquitoes was rapidly (that is, within one day) and strongly reduced by the Bti treatment, unlike the treatment with added community alone.

“Long-term” period (from day 1 to day 11 post-treatment with Bti)

In the “Bti” setup and after Bti treatment, the larval populations of mosquitoes showed a strong recolonisation in the first 8 days for *Anopheles* (“c” in Figure 1A) and the first 11 days for *Culex* (“c” in Figure 1B). In the “comBti” setup, no recolonisation was observed during this period for *Anopheles* (“c” in Figure 1A) and *Culex* (“c” in Figure 1B). In the “com” setup, the “long-term” period was characterised by a progressive decrease in the abundances of larvae of *Anopheles* spp. (“c” in Figure 1A) and *Culex* spp. (“c” in Figure 1B).

Moreover, the difference in larval abundances of *Anopheles* spp. was significant only between “Bti” and “comBti” in the whole “long-term” period (“c” in Figure 1A; ANOVA: P = 0.029, *Bonferroni* post hoc test: P = 0.032). The abundance of *Culex* spp. was higher in the setup “Bti” than in the setups “com” and “comBti” (“c” in Figure 1B); but this difference was not significant (ANOVA: P > 0.05).

A redundancy analysis (RDA) was carried out to investigate the influence of the density (expressed in number of individuals/L) and the composition of invertebrate communities on the recolonisation of ponds by mosquito larvae after Bti treatment. Only data from the “long-term” period (that is, from day 1 to day 11) of only the setups treated with Bti (that is, “Bti” and “comBti”), was used. The composition of the invertebrate communities (abundances of taxa) is reported in Table 2.

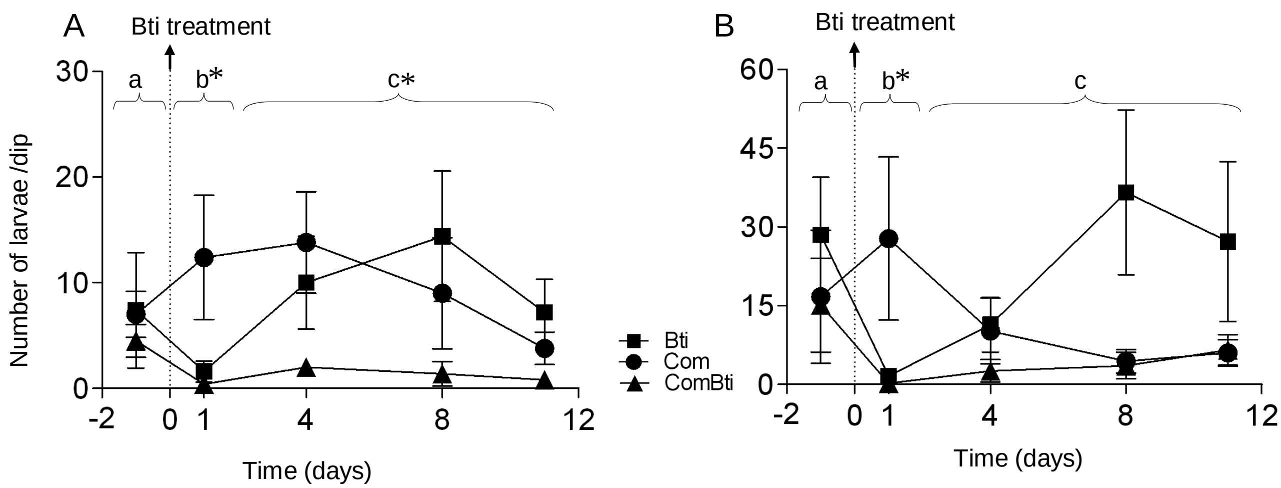

Figure 1. Variations of larval abundances of *Anopheles* spp. (A) and *Culex* spp. (B) in the "Bti and community" experiment. The different treatments are "Bti" for Bti alone, "Com" for added invertebrate community alone and "ComBti" for the treatment combining added invertebrate community and Bti. "a", "b", and "c" represent the "pre-treatment" period, and the "short-term" and "long-term" post exposure periods, respectively. * is the significance at P < 0.01.

Table 2. Mean and median values of the abundances (individual /L) of invertebrate taxa associated to mosquito larvae of all setups in "Bti and community" (n = 15, from day 0 to day 11) and "community density" (n = 16, from day 0 to day 15) experiments.

Parameter	Bti and community		Community density	
	Mean ± SE	Median	Mean ± SE	Median
Ceriodaphnia spp.	14 ± 5	3	25 ± 7	5
Chydorus spp.	3 ± 0.4	2	0.1 ± 0.1	0
Ciliata	16 ± 2	12	10 ± 1	8
Collembola	34 ± 2	1	3 ± 1	1
Cyclopoida	42 ± 4	36	38 ± 6	19
Hydra sp.	0.2 ± 0.1	0	0.4 ± 0.2	0
Hydracarina	0.2 ± 0.02	0	0.3 ± 0.1	0
L Chironomidae	8 ± 2	3	8 ± 2	3
L Dytiscidae	0.2 ± 0.1	0	0.2 ± 0.1	0
L Ephemeroptera	28 ± 3	24	33 ± 3	26
L Hydrophlilidae	0.2 ± 0.1	0	0.2 ± 0.1	0
L Odonata	4 ± 2	1	4 ± 1	3
Nauplii	13 ± 2	7	10 ± 4	3
Notonectidae	1.2 ± 0.2	1	2 ± 0.2	1
Ostracoda	4 ± 1	2	5 ± 1	1
Planaria	0.2 ± 0.1	0	0.2 ± 0.04	0
Rotifera	38 ± 16	1	100 ± 34	1

L: Larvae.

The predictor was the factor "Community density" with two levels; level "low density" for "Bti" treatment and level "high density" for "comBti" treatment. The results showed that, invertebrate taxa which were correlated with the first ordination axis had at least 22.5% of their variability associated with the factor "community density" (Figure 2, Monte Carlo permutation test: P = 0.032). Of these taxa, larvae of *Anopheles*, larvae of *Culex,* and nauplii were negatively correlated with the factor "community density" whereas *Chydorus* spp., Cyclopoida, Notonectidae, and larvae of Odonata were positively correlated (Figure 2). This result suggests that the recolonisation of ponds by mosquitoes was strongly reduced by a high density of associated invertebrate communities containing high

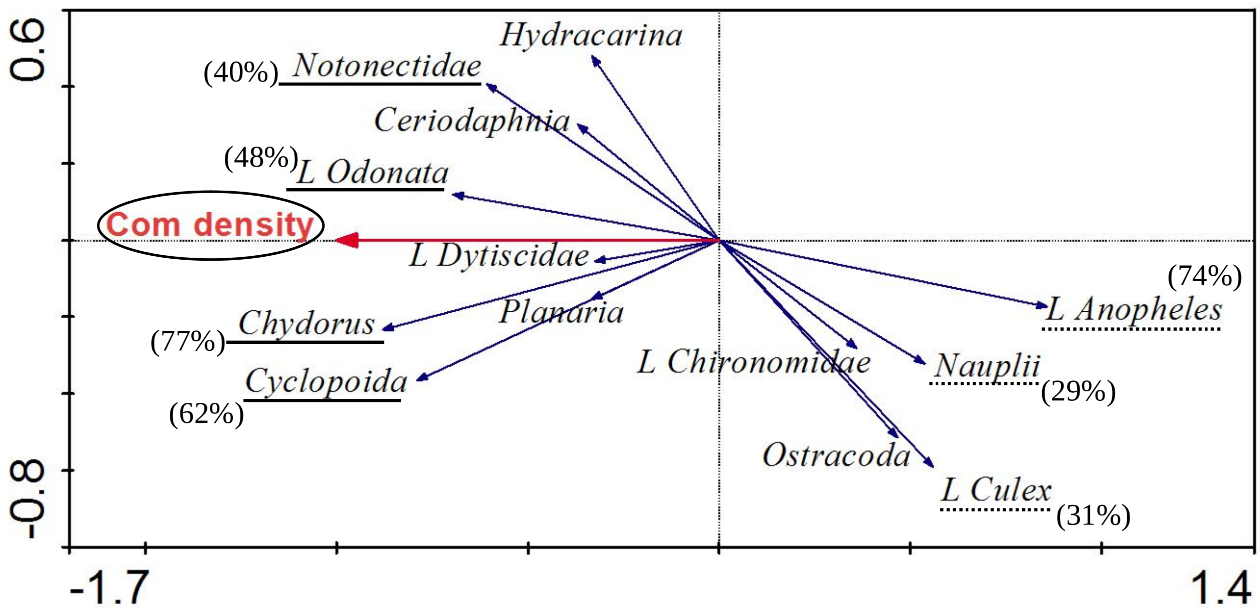

Figure 2. Ordination plot derived by the redundancy analysis (RDA) and showing the community structure of aquatic invertebrate taxa in relation to the factor community density (encircled), after Bti treatment in the "Bti and community" experiment. Underlined with a continuous line means significant ($P < 0.05$) and positive correlation with the community density; and underlined with a dashed line means significant ($P < 0.05$) and negative correlation with the community density. The percentages in parentheses represent the part of the variations associated to the community density for individual taxa. Only taxa which showed more than 10% of the associated variability with the first ordination axis are shown on this graph. "Com density" = Community density and "L.

abundances of *Chydorus* spp., Cyclopoida, Notonectidae, and larvae of Odonata.

The results of the Hotelling's T^2 test showed no significant difference between "com" and "comBti" setups ($P = 0.86$). This shows that Bti treatment did not change or impact the composition of the community associated to mosquito larvae.

"Community density" experiment

"Pre-treatment" period

During the pre-treatment period, larval abundances of the two species of mosquitoes were lower in the setup "high" community density than in the setups "low" and "medium" community density ("a" in Figure 3A for *Anopheles* spp.; and "a" in Figure 3B for *Culex* spp.). However this difference was not significant.

"Short-term" period (24 h following Bti treatment)

After 24 h following the treatment of ponds with Bti, there was a complete elimination of larvae of *Anopheles* spp. ("b" in Figure 3A) and *Culex* spp. ("b" in Figure 3B) in all setups. This result confirms the high efficiency of *Bacillus thuringiensis* var *israelensis* already observed in the "Bti and community" experiment, for controlling larval abundances of mosquitoes in a short time.

"Long-term" period (From days 1 to 15 after treatment with Bti)

This period was characterised by different patterns in the variations of larval abundance of mosquitoes between the setups "low", "medium", and "high" community density ("c" in Figure 3).

Indeed, three days following the treatment with Bti, the recolonisation of ponds by larvae of *Anopheles* spp. in the setups "low" and "medium" community density was effective ("c" in Figure 3A). In the setup "low" community density, a relatively high abundance of larvae of *Anopheles* spp. was maintained until day 7 and was followed by a progressive decrease until the end of the experiment ("c" in Figure 3A). In the setup "medium" community density, the abundance of larvae of *Anopheles* spp. decreased already from day 5 ("c" in Figure 3A). In the setup "high" community density, the abundance of larvae of *Anopheles* spp. remained the lowest during the whole post-treatment period. The differences in larval abundance of *Anopheles* spp. between the setups "low" and "high" community density

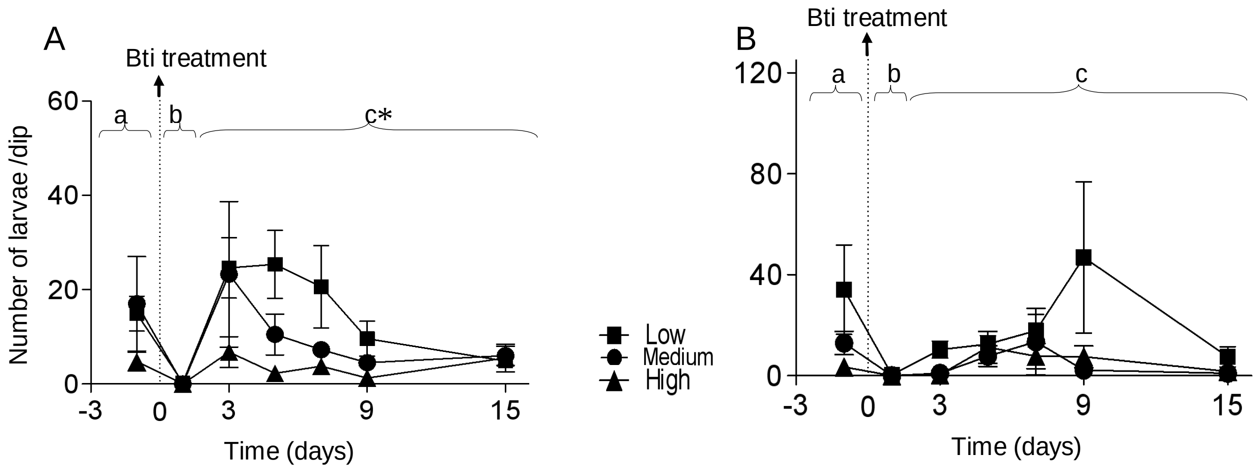

Figure 3. Variations of larval abundances of *Anopheles* spp. (A) and *Culex* spp. (B) in the “community density” experiment. “Low”, “Medium” and “High” represent the three treatments in terms of the density of added invertebrate communities. “a”, “b”, and “c” represent the “pre-treatment” period, and the “short-term” and “long-term” post exposure periods, respectively. * is the significance at $P < 0.01$.

were significant (“c” in Figure 3A; ANOVA: $P = 0.01$, *Dunnet-T* post hoc test: $P = 0.003$).

In the same period (that is, from days 1 to 15), larval abundances of *Culex* spp. were in general low in all setups (“c” in Figure 3B). However on day 9, a peak in larval abundance of *Culex* spp. was observed in the setup “low” community density (“c” in Figure 3B). This peak, which was not significant in comparison to the abundances of larvae in the setups “medium” and “high”, was followed by a fast decline on day 15 (“c” in Figure 3B).

We carried out a RDA to investigate the influence of the density and the composition of invertebrate communities on the recolonisation of ponds by mosquito larvae after Bti treatment; we used the data of “low”, “medium”, and “high” setups from the recolonisation period (from day 3 to 15). The composition of invertebrate communities (abundances of taxa) is reported in Table 2. The predictor was the factor “community density” with three levels: “low”, “medium”, and “high” community density. The outcomes revealed that the invertebrate taxa which were correlated with the first ordination axis had at least 22.8% of their variations explained by the factor “community density” (Figure 4; Monte Carlo permutation test: $P = 0.012$). Of these taxa, *Anopheles* larvae and Rotifera were negatively correlated with the factor community density whereas species of Cyclopoida, *Hydra* sp. and Ostracoda showed positive correlations with the community density (Figure 4). The other invertebrate taxa did not show significant correlations with the community density (Figure 4). However, the association between the abundance of larvae of *Culex* spp. and the factor “community density” was negative (Figure 4).

These results suggest that, the recolonisation of ponds by mosquito populations following the treatment with Bti was strongly negatively affected by the highest density of the community.

Compared sensitivity to associated aquatic invertebrate communities between larvae of *Anopheles* spp. and *Culex* spp.

In both experiments, *Anopheles* spp. was more affected by the factor community density than *Culex* spp. In the “Bti and community” experiment for example, 74% of the variations in the abundance of *Anopheles* spp. larvae were explained by the factor community density, versus 31% for larvae of *Culex* spp. (Figure 2). Similarly in the “community density” experiment, about 50% of the variations in the abundance of *Anopheles* larvae were associated to the community density while only a non-significant 15% explained variations occurred for *Culex* spp. larvae (Figure 4). This suggests a better potential control of larval populations of *Anopheles* spp. by the associated aquatic invertebrate communities than for *Culex* spp.

DISCUSSION

Liess and Duquesne (2014) proposed a method for larval control of mosquitoes in water bodies. This method combines two processes; one process is the suppression of pest larvae using an insecticide and, the other process is the prevention of the recolonisation of the larvae using biological agents such as competitors for food. In the present study, we implement this approach in the field, in human-made ponds of a tropical wetland area in Cameroon (Central Africa), a country with a high risk of

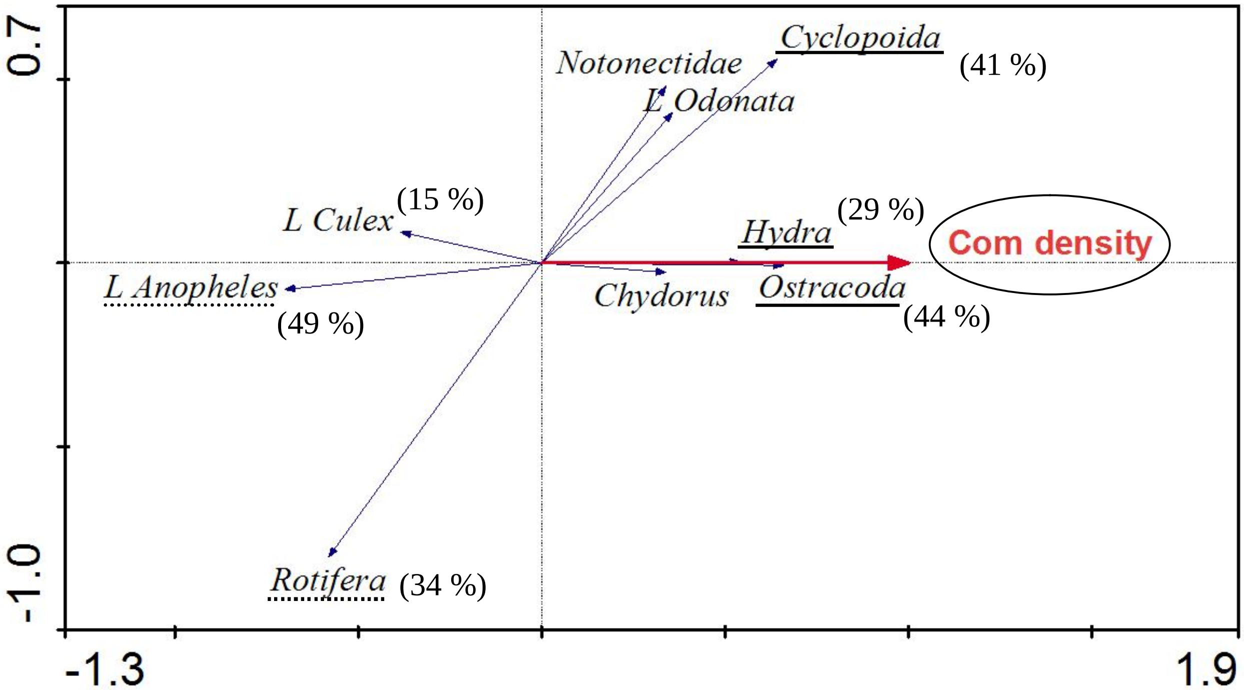

Figure 4. Ordination plot derived by the redundancy analysis (RDA) and showing the community structure of invertebrate taxa in relation to the factor community density (encircled) after Bti treatment in the "community density" experiment. Underlined with a continuous line means significant ($P < 0.05$) and positive correlation with the community density and underlined with a dashed line means significant ($P < 0.05$) and negative correlation with the community density. The percentages in parentheses nearby significant correlations represent the part of the variations associated to the community density for individual taxa. Only taxa which show more than 10% of the associated variability with the first ordination axis are exhibited on this graph. "Com density" = Community density and "L" = larvae.

mosquito borne diseases (e.g. malaria) (Sachs and Malaney, 2002).

Contribution of the present results to mosquito control

We observed that the use of Bti in combination with added community was more efficient for larval control of mosquitoes than the use of community alone, or Bti alone. The efficiency of this combined treatment increased with increasing density of invertebrate communities represented in this study by potential competitors of mosquito larvae such as Ostracoda and *Chydorus* spp., and predators such as Cyclopoida, *Hydra* sp., Notonectidae and larvae of Odonata.

The reinforcement of a Bti-based control of mosquito larvae (e.g. *Aedes aegypti*) by one invertebrate species (e.g. the Cyclopoida *Mesocyclops aspernicornis*) was shown in water storage containers in Thailand (Kosiyachinda et al., 2003). Besides, Kroeger et al. (2013) demonstrated a successful control of *Culex pipiens* using a combined action of Bti and natural competitors of mosquito larvae in Germany. There are different requirements for the use of a specific organism in mosquito control as did Kosiyachinda et al. (2003). This includes a good knowledge of its taxonomic classification, the particular species must be cultured successfully and survive the transfer to the field and then propagate. Moreover, using a functional group of species poses similar concerns: e.g., knowledge about the identification of the individual of that functional group. By contrast and in the present study, collecting invertebrate communities from neighbouring ponds formed earlier and thus richer in communities, as we did, has some advantages as none of the requirements cited above is required. Furthermore, the basis of the method proposed in this study is cost effective and also time and energy saving. The chance of success is higher in this case because taxa are broadly adapted to the environmental conditions of the area to be treated. Thus, our practice should be more easily implemented by neighbouring and non expert inhabitants of zones at high incidence of mosquito problems such as Mfou in Cameroon.

In tropical regions in general and in Cameroon in particular, temporary water bodies are generated spontaneously in the ground depressions in the onset of rainfalls. These ponds serve as additional breeding sites during the rainy seasons (Chaves and Koeenradt, 2010). Studies have shown that natural enemies (i.e. competitors and predators) can affect the abundances of larval populations of mosquitoes (Meyabeme Elono et al., 2010); through direct lethal effects on mosquito larvae (Knight et al., 2004; Marten and Reid, 2007; Duquesne et al., 2011) or/and through deterring oviposition by gravid females (Stav et al., 2000; Eitam et al., 2002; Mokany and Shine, 2003; Blaustein et al., 2004; Duquesne et al., 2011). Newly flooded ponds shelter low abundances of competitors and predators (Wilbur, 1997). Opportunistic organisms like many species of mosquitoes exploit preferentially such conditions to maximize the development success of their offspring (Mokany and Shine, 2003; Blaustein et al., 2004; Duquesne et al., 2011). This might explain the outbreaks of mosquito populations observed in tropical regions at the beginning of rainy seasons (Bigoga et al., 2007; Atangana et al., 2009). This study proposes a strategy that could be used in such conditions; that is, suppression of mosquito larvae using a biological pesticide (e.g. Bti) and anticipating the ageing of the communities of newly flooded ponds by adding invertebrate communities from older surrounding ponds. By so doing, the abundances of mosquito larvae after treatment will be limited and hopefully contributing in the prevention of outbreaks at the beginning of the rainy season.

Anopheles and *Culex* species: susceptibility to the treatments with Bti and high community densities

The larval populations of the two mosquitoes drastically decreased in all Bti treatments within 24 h after treatment. This efficacy of the VectoBac formulation of Bti has also been reported in other countries such as India (Amalraj et al., 2000), Kenya (Kahindi et al., 2008), Australia (Russell et al., 2003), Turkey (Aldemir 2007) and Germany (Kroeger et al., 2013). Similarly, other formulations of Bti such as Bactimos and Teknar, were successfully used for controlling vector mosquitoes (Poopathi and Tyagi, 2006). Owing to the fact that larval development of mosquitoes generally last only few days (e.g. seven days, Mokany and Shine, 2003), fast acting larvicides, such as Bti, are indeed a good tool for mosquito control strategies.

Anopheles species were affected more strongly by high community density than *Culex* species. It was revealed that some of the predators (e.g. Cyclopoida) that had a positive correlation with the community density during the present study preyed substantially more on larvae of *Anopheles* than on larvae of *Culex* (Marten and Reid, 2007). Therefore, the higher susceptibility of populations of *Anopheles* may be a consequence of prey preference behaviour by such predators. Although we could not explicitly separate the relevance of the direct lethal effect due to natural enemies on mosquito larvae from the oviposition deterring behaviour of female mosquitoes, the expected role of invertebrate community in preventing the recolonisation of ponds by mosquitoes after a treatment with Bti was demonstrated in this study.

CONCLUSION

Our results suggest that natural invertebrate communities had a great potential in improving the larval control of mosquitoes by Bti-based VectoBac. So, owing to the fact that newly flooded ponds contribute in increasing the abundance of mosquitoes, including disease vectors such as *Anopheles* species, we recommend the combination of Bti and local invertebrate communities for larval control of this pest, especially during the first weeks of therainy seasons.

ACKNOWLEDGEMENTS

We would like to thank K. Foit for helping with statistical analysis, T. Njine, C.F. Bilong Bilong, S. Zebaze Togouet for their active participation in the establishment of the collaboration between the Helmholtz Centre for Environmental Research-UFZ Leipzig (Germany) and the laboratory of General Biology of the University of Yaounde I (Cameroon). We are grateful to P. Ywuoung, R. Kayo, J. Nzieleu, H. Tsila, and N.F. Meyong for field assistance; and inhabitants of Mfou subdivision for their enthusiastic adoption of this project. This work was supported financially by DAAD (German Academic Exchange Service) and the Helmholtz Centre for Environmental Research-UFZ Leipzig, Germany.

REFERENCES

Aldemir A, **2007**. The Efficacy and longevity of Vectobac® 12 AS and Vectobac® G (both based on *Bacillus thuringiensis* subsp. *israelensis*) for the control of mosquitoes in Turkey. Turk J Zool, 31:317-323.

Amalraj DD, Sahu SS, Jambulingam P, Boopathi Doss PS, Kalyanasundaram M, Das PK, **2000**. Efficacy of aqueous suspension and granular formulations of *Bacillus thuringiensis* (Vectobac) against mosquito vectors. Acta Tropica, 75:243-246.

Atangana J, Fondjo E, Fomena A, Tamesse J L, Patchoké S, Ndjemaï HNM, Bessong Ndong PA, **2009**. Seasonal variations of malaria transmission in Western Cameroon highlands: Entomological, parasitological and clinical investigations. J Cell Anim Biol, 3(3):33-38.

Barbazan P, Baldet T, Darriet F, Escaffre H, Haman Djoda D, Hougard JM, **1997**. Control of Culex quinquefasciatus (Diptera: Culicidae) with Bacillus sphaericus in Maroua, Cameroon. J Am Mosq Control Assoc, 13(3):263-269.

Becker N, Petrić D, Zgomba M, Boase C, Dahl C, Lane J, Kaiser A, **2003**. Mosquitoes and their control. New York, NY: Kluwert Academic/Plenum Publishers, pp.498.

Bigoga JD, Manga L, Titanji VPK, Coetzee M, Leke RGF, **2007**. Malaria vectors and transmission dynamics in coastal south-western Cameroon. Malar J, 6:5-17.

Blaustein L, Kiflawi M, Eitam A, Mangel M, Cohen JE, **2004**. Oviposition habitat selection in response to risk of predation in temporary pools: mode of detection and consistency across experimental venue. Oecologia, 138:300-305.

Boisvert M, **2005**. Utilization of Bacillus thuringiensis var. israelensis (Bti)-based formulations for the biological control of mosquitoes in Canada. 6th Pacific Rim Conference on the biotechnology of *Bacillus thuringiensis* and its environmental impact, Oct 30-Nov 3, Victoria BC. pp: 87-93.

Boisvert M, **Boisvert** J, **2000**. Effect of *Bacillus thuringiensis* var. *israelensis* on target and nontarget organisms: a review of laboratory and field experiments. Biocontrol Sci Technol, 10:517-561.

Camargo EP, **2008**. Tropical diseases. Estudos Avançados, 22(64):95-110.

Chaves LF, **Koenraadt** CJM, **2010**. Climate change and highland malaria: fresh air for a hot debate. Q Rev Biol, 85(1):27-55.

Duquesne S, Kroeger I, Kutyniok M, Liess M, **2011**. The potential of Cladocera as controphic competitors of the mosquito *Culex pipiens*. J Med Entomol, 48(3):554-560.

Durand JR, **Lévêque** C, **1980**. Aquatic flora and fauna of sahelo-Sudanese Africa. Volume I. Paris: Office for Overseas Scientific and Technical Research (O.R.S.T.O.M). pp: 873.

Eitam A, Blaustein L, Mangel M, **2002**. Effects of *Anisops sardea* (Hemiptera : Notonectidae) on oviposition habitat selection by mosquitoes and other dipterans and on community structure in artificial pools. Hydrobiologia 485:183-189.

Faye O, Konate L, Mouchet J, Fontenille D, Ngayo Sy S, Hebrard G, Herve JP, **1997**. Indoor resting by outdoor biting females of *Anopheles gumbiue* complex (Diptera: Culicidae) in the Sahel of Northern Senegal. J Med Entomol, 34(3):285-289.

Fontenille D, **Carnevale** P, **2006**. Malaria and dengue vector biology and control in West and Central Africa. In: Bridging laboratory and field research for genetic control of disease vectors, Knols BGJ, Louis C, (Eds.). Dordrecht: Springer, pp: 91-99.

Gotelli NJ, **Ellison** AM, **2004**. A primer of ecological statistics. Sinauer Associates Inc., Sunderland, MA. p: 492.

Hemingway J, **Ranson** H, **2000**. Insecticide resistance in insect vectors of human disease. Ann Rev Entomol, 45:371-391.

Hougard JM, **Back** C, **1992**. Perspective on the bacterial control of Vectors in the tropics. Parasitol Today, 8(11):364-366.

Kahindi SC, Midega JT, Mwangangi JM, Kibe LW, Nzovu J, Luethy P, Githure J, Mbogo C M, **2008**. Efficacy of Vectobac dt and CulinexCombi against mosquito larvae in unused swimming pools in Malindi, Kenya. J Am Mosq control Assoc, 24(4):538--542.

Knight TM, Chase JM, Goss CW, Knight JJ, **2004**. Effects of interspecific competition, predation and the interaction on survival and development time of immature *Anopheles quadrimaculatus*. J Vector Ecol, 29(2):277-284.

Kosiyachinda P, Bhumiratana A, Kittayapong P, **2003**. Enhancement of the efficacy of a combination of *Mesocyclops aspericornis* and *Bacillus thuringiensis* var. *israelensis* by community-based products in controlling *Aedes aegypti* larvae in Thailand. Am J Trop Med Hyg, 69(2):206-212.

Kroeger I, Liess M. Dziock F, Duquesne S, **2013**. Sustainable control of mosquito larvae in the field by the combined actions of the biological insecticide Bti and natural competitors. J Vector Ecol, 38(1):82-89.

Kumar R, **Hwang** JS, **2006**. Larvicidal efficiency of aquatic predators: a perspective for mosquito biocontrol. Zool Stud, 45 (4): 447-466.

Lacey LA, **2007**. *Bacillus thuringiensis serovariety israelensis* and *Bacillus sphaericus* for mosquito control. J Am Mosq Control Assoc, 23(2):133-163.

Leps J, **Smilauer** P, **2003**. Multivariate analysis of ecological data using CANOCO. Cambridge University Press, Cambridge. pp: 38-43.

Liess M, **Duquesne** S, **2014**. Method for the eradication of pathogenic microorganisms in an aqueous system. Patent US8,915,013 B2

Marten GG, **Reid** JW, **2007**. Cyclopoid copepods. J Am Mosq Control Asso, 23:65-92.

Mokany A, **Shine** R, **2003**. Oviposition site selection by mosquitoes is affected by cues from conspecific larvae and anuran tadpoles. Austral Ecol, 28:33-37.

Meyabeme Elono AL, Liess M, Duquesne S, **2010**. Influence of competing and predatory invertebrate taxa on larval populations of mosquitoes in temporary ponds of wetlands areas in Germany. J Vector Ecol, 35(2):419-427.

Mutuku F, Alaii MJA, Bayoh MN, Gimnig JE, Vulule JM, Walker ED, kabiru E, Hawley WA, **2006**. Distribution, description, and local knowledge of larval habitats of *Anopheles gambiae* s.l. in a village in Western Kenya. Am J Trop Med Hyg, 74(1):44-53.

Oyewole IO, Awololab TS, Ibidapoc CA, Oduolad AO, Okwac OO, Obansad JA, **2007**. Behaviour and population dynamics of the major anopheline vectors in a malaria endemic area in southern Nigeria. J Vector Borne Dis, 44:56-64.

Poopathi S, **Tyagi** BK, **2006**. The challenge of mosquito control strategies: from primordial to molecular approaches. Biotechnol Mol Biol Rev, 1(2):51-65.

Ramirez JL, Garver LS, Dimopoulos G, **2009**. Challenges and Approaches for mosquito targeted malaria control. Curr Mol Med, 9:116-130.

Ross R, **1897**. On some peculiar pigmented cells found in two mosquitos fed on malarial blood. Br Med J, 2(1929):1786-1788.

Russell TL, Brown MD, Purdie DM, Ryan PA, Kay BH, **2003**. Efficacy of VectoBac (*Bacillus thuringiensis* variety *israelensis*) formulations for mosquito control in Australia. J Econ Entomol, 96(6):1786-1791.

Sachs J, **Malaney** P, **2002**. The economic and social burden of malaria. Nature, 415: 680-685.

SCOR/UNESCO, **1966**. Determination of Photosynthetic Pigments in Seawater. Monographs on Oceanographic Methodology. Vol 1. UNESCO, Paris, pp: 11-18.

Simard F, Ayala D, Kamdem GC, Pombi M, Etouna J, Ose K, Fotsing J-M, Fontenille D, Besansky NJ, Costantini C, **2009**. Ecological niche partitioning between *Anopheles gambiae* molecular forms in Cameroon: the ecological side of speciation. BMC Ecology, 9:17.

Stav G, Blaustein L, Margalit J, **2000**. Influence of nymphal Anax imperator (Odonata: Aeshnidae) on oviposition by the mosquito *Culiseta Longiareolata* (Diptera:Culicidae) and community structure in temporary pools. J Vector Ecol, 25:190-202.

Tachet H, Richoux P, Bournaud M, Usseglio-Polatera P, **2003**. Invertébrés d'eau douce, systematique, biologie, ecologie. CNRS, Paris. pp: 62-351.

ter Braak CJF Smilauer P, **2003**. Canoco for Windows Version 4.52. Biometrics-Plant Research International, Wageningen. pp: 174-183.

Tuno N, Kjaerandsen J, Badu K, Kruppa T, **2010**. Blood-feeding behaviour of *Anopheles gambiae* and *Anopheles melas* in Ghana, Western Africa. J Med Entomol, 47(1):28-31.

Ward HB, **Whipple** GC, **1959**. Fresh-water biology. 2nd Ed. New York, London: John Wiley & Sons Inc, pp: 1248.

WHO [World Health Organization], **1975**. Manual on practical entomology in Malaria. Part II: methods and techniques. World Health Organization Offset Publication No. 13, Geneva. Pp: 55-60.

Wilbur HM **1997**. Experimental ecology of food webs: complex systems in temporary ponds. Ecology, 78(8):2279-2302.

Citation: Meyabeme Elono AL, Liess M, Duquesne S, 2016. Invertebrate communities improve the efficiency of the biological pesticide *Bacillus thuringiensis* var. *israelensis* (Bti) in mosquito control: A case study in Cameroon, Central Africa. Int J Ecol Ecosolution, 3(3): 30-39.