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Factors influencing the toxicity of xenobiotics against larval mosquitoes

Facteurs influençant la toxicité des xénobiotiques sur les larves de moustiques

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Abstract

In order to examine the factors influencing xenobiotic toxicity against larval mosquitoes, the larvicidal performances of two conventional insecticides (temephos and *Bacillus thuringiensis* var. *israelensis*: *Bti*) and a new potential phyto-insecticide (decomposed leaf litter) were compared under different conditions against three detritivorous larval mosquito types. Bioassays performed under standard conditions indicated differential tolerance levels according to the xenobiotic and the larval type. Bioassays performed under different conditions of xenobiotic dose and geometry of the water column indicated differential effects of those parameters on mortality rates. This allowed us to distinguish the performances of temephos versus those of *Bti* and leaf litter. These toxicological performances were examined as indicators for analysis of xenobiotic bioavailability for mosquito larvae in environmental water, and also for their comparative interest in field mosquito control. ***To cite this article: D. Rey et al., C. R. Biologies 326 (2003).***

Résumé

Afin d'examiner l'importance relative des paramètres influençant la toxicité des xénobiotiques sur les larves de moustiques, les effets larvicides de deux insecticides conventionnels (téméphos et *Bacillus thuringiensis* var. *israelensis* : *Bti*) et d'un nouveau phyto-insecticide potentiel (litière arborescente décomposée) sont comparés sur trois espèces de moustiques. Des bio-essais en conditions standard montrent une tolérance larvaire différant selon le type de xénobiotique et de larve. Des bio-essais sous différentes conditions de dose de xénobiotique et de géométrie de la colonne d'eau mettent en évidence des effets différents sur la mortalité larvaire, les effets du téméphos étant opposables à ceux du *Bti* et de la litière. Ces performances sont examinées en tant qu'indicateurs dans l'analyse de la biodisponibilité des xénobiotiques pour les larves de moustiques dans le milieu aquatique et pour comparer l'intérêt stratégique de ces différents larvicides. ***Pour citer cet article : D. Rey et al., C. R. Biologies 326 (2003).***

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Mots-clés : litière toxique ; insecticides conventionnels ; paramètres environnementaux ; biodisponibilité ; larves de moustiques ; lutte biologique

Version française abrégée

Dans les gîtes à moustiques subalpins, les litières arborescentes présentent une toxicité différentielle sur les larves détritivores, grâce à des complexes lignine–polypeptide formés au cours de la décomposition foliaire. Ce nouveau type de larvicide pourrait être utilisé dans une nouvelle stratégie de démoustication visant à remédier aux nombreux problèmes engendrés par l'usage généralisé des insecticides conventionnels. L'intérêt de cette stratégie est évalué par rapport aux stratégies conventionnelles, en comparant les propriétés larvicides de la litière décomposée d'aulne brute à celles du téméphos (insecticide organophosphoré) et de *Bacillus thuringiensis* var. *israelensis* (*Bti*) (bactério-insecticide) sur trois modèles différents de larves de moustiques détritivores (*Aedes albopictus* Skuse, *Culex pipiens* L. et *Anopheles stephensi* Liston).

Une comparaison par bio-essais effectués en conditions standard montre une forte toxicité du téméphos par rapport au *Bti* et à la litière décomposée, et une tolérance particulière de chaque modèle larvaire en fonction du type de larvicide. Une comparaison par bio-essais effectués dans différentes conditions de dose de larvicide (concentration) et de géométrie de la colonne d'eau (surface, volume, hauteur) met en évidence des effets différents de ces paramètres sur la mortalité, selon le type de larvicide et/ou de larve. Pour la litière décomposée, on observe un effet de surface significatif pour *Ae. albopictus* et *Cx. pipiens*, ainsi qu'un effet de volume significatif pour *An. stephensi*. Pour le *Bti*, on observe un effet de volume significatif pour *An. stephensi* et *Cx. pipiens*. Pour le téméphos, on observe des effets de surface et de volume significatifs, opposant *An. stephensi* à *Cx. pipiens* et *Ae. albopictus*.

Ces différences entre performances toxiques, selon le type de bio-essai, de larvicide et de modèle larvaire, montrent la complexité des interactions entre les différents facteurs intervenant dans la biodisponibilité du xénobiotique par rapport à sa cible. Au niveau du xénobiotique, le comportement physique du larvicide dans l'eau interfère avec la géométrie du milieu : on distingue les effets du téméphos, soluble dans l'eau, de ceux du *Bti* et de la litière, formant des suspensions plus ou moins stables dans l'eau. Au niveau de la cible, le positionnement dans la colonne d'eau et le comportement alimentaire de chaque modèle larvaire interfèrent avec la répartition du larvicide dans le milieu : on oppose *An. stephensi*, filtreur–collecteur extensif de surface, à *Cx. pipiens*, filtreur–collecteur intensif de pleine eau, et à *Ae. albopictus*, brouteur–collecteur intensif de fond. La complexité de ces interactions, révélée dans notre système expérimental simplifié, doit s'accentuer sur le terrain.

L'ensemble des résultats de notre comparaison montre l'intérêt potentiel d'une stratégie « litière décomposée » en démoustication, notamment dans la gestion durable d'espèces anthrophiles vectrices potentielles (*Culex*, *Aedes*). Aux arguments toxicologiques s'ajoutent des arguments environnementaux, biologiques et opérationnels, la litière décomposée contenant naturellement à la fois la substance active et l'adjuvant, nécessaires à toute formulation insecticide. Au-delà de son intérêt appliqué, notre étude révèle la complexité des facteurs intervenant dans l'écologie nutritionnelle et toxicologique des larves de moustiques en milieu aquatique.

1. Introduction

The extensive and widespread use of synthetic insecticides has caused some concerns regarding the toxicological, economical and environmental impact of those xenobiotics [1]. Because of these concerns, some search for new insecticide strategies is now being conducted, particularly on natural plant products, in order to develop phytochemicals possessing efficient, environmentally safe and target-specific pesticidal activity [2,3].

Among these phytochemicals, polyphenols are known for their dietary toxicity to phytophagous insects [4] and detritivorous larval culicine taxa [5].

Particular involvement of polyphenolic complexes in plant-larval mosquito dietary interactions was recently evidenced in the subalpine mosquito breeding sites [6]. These insoluble complexes, included within the cell-wall fraction of crude ten-month-decomposed arborescent leaf litter, exert a dietary toxicity against some detritivorous larval culicine taxa [7, 8]. Once ingested, these xenobiotics wreck the larval midgut epithelium [9] following a lysis pattern similar to that observed after intoxication with the bacterio-insecticide *Bacillus thuringiensis* ssp. *israelensis* (*Bti*) [10]. Preliminary phytochemical characterizations have revealed that these larvicidal molecules are related to lignin–polypeptidic complexes [11–13]. Comparative toxicological and behavioural investigations have suggested that the dietary use of decomposed arborescent leaf litter may constitute a new larvicidal strategy usable against anthropophilic vector-competent invasive species resistant to conventional insecticides [14,15].

The efficacy of any mosquito larvicide may, however, depend upon its bioavailability for target larvae in the environmental water together with the own tolerance level of each larval taxon. Several environmental parameters may influence the performances of a given larvicide [16,17]. Contrary to a water-soluble larvicide like temephos, which is used under homogeneous solutions without any dietary interest [18], decomposed leaf litter and *Bti* are used under heterogeneous suspensions with a dietary interest [15,19]. The physical heterogeneity of these dietary larvicicides may interfere with their toxicity, because of possible modifications in their bioavailability to target larvae in the water column. These modifications may occur according to the dose of toxic material and the geometric characteristics of the water column, which interfere with the own larval feeding behaviour.

This is why, in this paper, we examined the larvicidal performances of toxic leaf litter in comparison with those of temephos and *Bti* in different conditions of xenobiotic dose and geometry of the environmental water column, liable to simulate field conditions. Such a comparative investigation was performed against different taxa representative of the main detritivorous mosquito larval feeding behaviours [20]. Our results will help us to predict the real efficacy of leaf litter versus conventional larvicides in different field conditions. Moreover, nutritional ecology of mosquito larvae is poorly understood, because resulting from the complex interaction between numerous abiotic and biotic parameters [21]. The different performances of the xenobiotics used in this comparative study under various environmental conditions may serve as indicators liable to evidence the different parameters involved in xenobiotic bioavailability for mosquito larvae in the aquatic medium.

2. Materials and methods

2.1. Animals

Aedes albopictus Skuse, *Culex pipiens* L., and *Anopheles stephensi* Liston are widespread anthropophilic vector-competent mosquitoes, whose larvae are detritivorous non-selective feeders. *Ae. albopictus* is collecting-gathering in loose deposits on the substratum, while *Cx. pipiens* and *An. stephensi* are collecting–filtering particulate food in suspension respectively in the main body and at the surface of the water column [20]. In order to obtain reproducible results, experimentation was performed using calibrated third instar larvae from laboratory strains (*Ae. albopictus*: Italian strain [22]; *Cx. pipiens*: S-LAB strain [23]; *An. stephensi*: ST15 strain from 'Institut Pasteur', Paris).

2.2. Larvicidal materials

Temephos (Abate 500 E) was used in aqueous solutions. *Bti* (Bactimos WP, 6000 AAU mg^{-1}, and natural crude ten-month decomposed alder leaf litter were used in aqueous suspensions. *Bti* particle size did not exceed 50 µm [24]. Leaf litter (average particle size: 0.4 mm) was sampled and prepared following David et al. [9]. All larvicidal media were made with pure tap water adjusted to pH 7.5 according to Rey et al. [15].

2.3. Standard bioassays

These three larvicidal materials were toxicologically evaluated against the three larval types using the standard bioassay technique [25] performed in single-use vials containing 50 ml aqueous media. Temephos was used at concentrations varying from 0.0004 to

Table 1
Different experimental parameters selected in the modified bioassays

Bioassay number	Experimental parameters			
	Geometry of the water column			Dose of toxic material
	Surface	Volume	Height	Concentration
1	S_2	V_1	H_1	C_3
2	S_2	V_1	H_1	C_4
3	S_2	V_2	H_2	C_1
4	S_2	V_2	H_2	C_2
5	S_1	V_1	H_3	C_3
6	S_1	V_1	H_3	C_4
7	S_1	V_2	H_4	C_1
8	S_1	V_2	H_4	C_2

Table 2
Doses (expressed as concentrations) of toxic material and times of exposition used in the modified bioassays

Type of toxic material	Dose of toxic material (mg l^{-1})				Time of exposition (h)							Larval types
	C_1	C_2	C_3	C_4	T_1	T_2	T_3	T_4	T_5	T_6	T_7	
					2	4						*Ae. albopictus*
Leaf litter	25	50	100	200			5	6				*Cx. pipiens*
					2	4						*An. stephensi*
									17	22	24	*Ae. albopictus*
Bti	0.0375	0.0750	0.150	0.300					17	22	24	*Cx. pipiens*
									17	22	24	*An. stephensi*
									17	22	24	*Ae. albopictus*
Temephos	0.0009	0.0018	0.0036	0.0072	2	4	5					*Cx. pipiens*
									17	22	24	*An. stephensi*

0.02 mg l^{-1}. *Bti* was used at concentrations varying from 0.05 to 50 mg l^{-1}. Leaf litter was used at concentrations varying from 20 to 7000 mg fresh mass per litre. Each bioassay was conducted in triplicate at 25 °C, on 20 starved larvae, during 24 h. Controls were reared in pure tap water with pH adjusted as above.

2.4. Modified bioassays

Different parameters susceptible to interfere with the larval mortality were examined for each toxic material throughout different bioassay designs. Those parameters were: dose (expressed as concentration) of toxic material (four concentrations assayed: $C_1 < C_2 < C_3 < C_4$, see Tables 1, 2); volume of the water column (two volumes assayed: $V_1 = 50$ ml $< V2 = 200$ ml); surface of the water column (two surfaces assayed: $S_1 = 10$ cm^2 $< S_2 = 60$ cm^2); height of the water column (four heights assayed: $H_1 = 1$ cm $< H_2 = 3.5$ cm $< H_3 = 5$ cm $< H_4 = 20$ cm). One parameter varying for one bioassay to another, eight different bioassay designs for each toxic material were selected (Table 1). Because of the differential toxicity levels of the bioassayed materials, mortality ranges suitable for a comparable dose-mortality correlation among the three larval types were preliminary adjusted according to the dose of toxic material and the time of exposition. This allowed us to adopt the dose and time parameters indicated in Table 2.

2.5. Data analysis

After correction with Abbott's formula [26], mortality data were analysed with the log-probit program of Raymond [27]. Efficacy of the different parameter combinations on the mortality rate of each larval type for each toxic material were assessed by a repeated measure analysis of variance using GLM procedure [28]. In this study, between-subject effects were: dose (expressed as concentration: C), surface (S), and volume (V). Within-subject effects were the above effects with the additional effects of time (T).

Table 3
Comparative larval tolerance to leaf litter, *Bti* and temephos among the three larval types, obtained with standard bioassays. Mortality rates are expressed as mean 24-h median lethal concentration (LC_{50}) values

	24 h median lethal concentration (mg/l)		
	LC_{50}	95% confidence limits of LC_{50}	Slope ± SE
Leaf litter			
Ae. albopictus	35.31	28.75–42.82	2.67 ± 0.34
An. stephensi	22.36	11.1–33.54	1.35 ± 0.21
Cx. pipiens	11.98	1.35–25.78	1.24 ± 0.32
Bti			
An. stephensi	0.18	0.14–0.23	2.80 ± 0.68
Cx. pipiens	0.12	0.11–0.14	7.60 ± 2.55
Ae. albopictus	0.04	0.02–0.08	2.89 ± 0.95
Temephos			
An. stephensi	0.15	NC	1.31 ± 0.72
Cx. pipiens	0.07	NC	0.64 ± 0.39
Ae. albopictus	0.03	0.006–0.14	0.94 ± 0.58

NC = not computable.

3. Results

3.1. Differential larvicidal effects of leaf litter, Bti *and temephos in standard conditions*

Standard bioassays allowed us to evidence a differential tolerance of *Ae. albopictus*, *Cx. pipiens* and *An. stephensi* against leaf litter, *Bti* and temephos, according to both the toxic material and the larval type (Table 3). Leaf litter was less toxic than conventional insecticides, and temephos was more toxic than *Bti*. When using leaf litter, *Cx. pipiens* was the most sensitive, followed by *An. stephensi* and *Ae. albopictus*. When using conventional insecticides, *An. stephensi*, relatively less sensitive to *Bti* and temephos, may be opposed to *Ae. albopictus* and *Cx. pipiens*, relatively more sensitive.

3.2. Influence of dose of toxic material, time of exposition and environmental geometrical parameters on the larvicidal effects of leaf litter, Bti*, and temephos*

Mortality data obtained throughout the 8 modified bioassay designs showed that, whereas dose and time effects were always significant, the effects of the other parameters varied according to the toxic material and the larval type (Table 4, Figs. 1–3).

Leaf-litter larvicidal effect appeared to be significantly influenced by the water surface for *Ae. albopictus* and *Cx. pipiens*, the mortality rates decreasing with the surface of the water column (Fig. 1a). On the other hand, the volume effect was significant for *An. stephensi*, the mortality rates decreasing with the volume (i.e., height) of the water column (Fig. 1b). Significant surface × volume and dose × volume interactions were found for *Ae. albopictus* (Figs. 1b and 1c). Conversely, a significant concentration × surface interaction was found for *An. stephensi* and *Cx. pipiens* (Fig. 1e).

Bti larvicidal effect appeared to be significantly influenced by the environmental volume only for *An. stephensi* and *Cx. pipiens*, the mortality rates decreasing with the volume (Fig. 2a). A significant surface × volume interaction was also observed for *An. stephensi* (Fig. 2b).

Temephos larvicidal effect appeared to be significantly influenced by the environmental surface and volume, whatever the dose and the larval type, with opposite effects on *An. stephensi* versus *Ae. albopictus* and *Cx. pipiens* (Fig. 3a). On the contrary, the volume (or height) effect allowed us to oppose *Cx. pipiens* and *An. stephensi* versus *Ae. albopictus* (Fig. 3b).

4. Discussion

Results from our different bioassay designs reveal that numerous intrinsic and extrinsic factors may influence the toxicological performances of a given insecticide against a given target mosquito larva.

Table 4
Analysis of variance for the effects on the larval mortality of dose of larvicidal material (expressed as concentration), environmental geometrical parameters (surface, volume) and time, using toxic leaf litter (a), *Bti* (b), and temephos (c). Significant results at $P < 0.05$ are in bold

Source	*Aedes albopictus*			*Anopheles stephensi*			*Culex pipiens*		
	dF	F value	$P > F$	dF	F value	$P > F$	dF	F value	$P > F$
(a) Leaf litter									
Dose (C)	1	15.66	**0.0010**	1	15.35	**0.0018**	1	20.87	**0.0004**
Surface (S)	1	89.67	**< 0.0001**	1	0.87	0.3691	1	44.35	**< 0.0001**
Volume (V)	1	0.45	0.5101	1	30.60	**< 0.0001**	1	0.09	0.7689
$C \times S$	1	2.62	0.1242	1	4.92	**0.0449**	1	33.11	**< 0.0001**
$C \times V$	1	4.97	**0.0396**	1	1.59	0.2299	1	0.69	0.4211
$S \times V$	1	13.77	**0.0017**	1	4.14	0.0629	1	0.02	0.8902
$C \times S \times V$	1	23.62	**0.0001**	1	2.43	0.1433	0		
Error	17			13			14		
Time (T)	1	200.37	**< 0.0001**	1	178.24	**< 0.0001**	1	42.11	**< 0.0001**
$T \times C$	1	0.81	0.3801	1	0.04	0.8499	1	6.35	**0.0245**
$T \times S$	1	0.09	0.7636	1	0.26	0.6205	1	16.81	**0.0011**
$T \times V$	1	7.85	**0.0123**	1	1.31	0.2732	1	0.17	0.6883
$T \times C \times S$	1	13.37	**0.0020**	1	3.82	0.0725	1	14.45	**0.0019**
$T \times C \times V$	1	13.45	**0.0019**	1	3.93	0.0690	1	0.00	0.9635
$T \times S \times V$	1	0.79	0.3858	1	0.24	0.6321	1	0.48	0.4984
$T \times C \times S \times V$	1	6.08	**0.0246**	1	0.13	0.7231	0		
Error	17			13			14		
(b) *Bti*									
Dose (C)	1	14.54	**0.0015**	1	49.99	**< 0.0001**	1	19.33	**0.0005**
Surface (S)	1	3.50	0.0796	1	0.58	0.4564	1	0.13	0.7186
Volume (V)	1	0.01	0.9172	1	12.46	**0.0030**	1	115.80	**< 0.0001**
$C \times S$	1	4.02	0.0622	1	0.60	0.4519	1	0.73	0.4045
$C \times V$	1	0.12	0.7361	1	0.41	0.5329	1	2.83	0.1117
$S \times V$	1	0.16	0.6955	1	9.35	**0.0080**	1	0.45	0.5115
$C \times S \times V$	1	0.15	0.7026	1	0.02	0.8991	1	0.76	0.3600
Error	16			15			16		
Time (T)	2	4.82	**0.0148**	2	11.72	**0.0002**	2	85.18	**< 0.0001**
$T \times C$	2	3.08	0.0597	2	4.20	0.2460	2	5.21	**0.0110**
$T \times S$	2	0.90	0.4171	2	1.51	0.2374	2	0.94	0.4000
$T \times V$	2	0.67	0.5207	2	0.69	0.5088	2	23.34	**< 0.0001**
$T \times C \times S$	2	1.18	0.3214	2	2.72	0.0821	2	2.99	0.0644
$T \times C \times V$	2	0.45	0.6414	2	1.39	0.2657	2	1.72	0.1957
$T \times S \times V$	2	1.20	0.3146	2	1.84	0.1767	2	0.27	0.7678
$T \times C \times S \times V$	2	2.31	0.1155	2	0.64	0.5332	2	1.53	0.2316
Error	32			30			32		
(c) Temephos									
Dose (C)	3	85.94	**< 0.0001**	3	14.69	**< 0.0001**	3	9.17	**0.0005**
Surface (S)	1	26.14	**< 0.0001**	1	12.16	**0.0033**	1	18.62	**0.0005**
Volume (V)	1	192.81	**< 0.0001**	1	21.41	**0.0003**	1	42.09	**< 0.0001**
$S \times V$	1	0.10	0.7590	1	0.04	0.8533	1	0.51	0.4853
$C \times S \times V$	4	74.18	**< 0.0001**	4	16.12	**< 0.0001**	4	2.06	0.1345
Error	23			15			16		
Time (T)	2	10.74	**0.0001**	2	22.59	**< 0.0001**	2	15.70	**< 0.0001**
$T \times C$	6	2.05	0.0748	6	2.47	**0.0409**			
$T \times S$	2	2198.00	**< 0.0001**	2	0.20	0.8211	2	0.07	0.9314
$T \times V$	2	10.45	**0.0002**	2	1.49	0.2410	2	8.04	**0.0015**
$T \times S \times V$	2	14.60	**< 0.0001**	2	2.11	0.1393	2	8.48	**0.0011**
$T \times C \times S \times V$	8	2.93	**0.0099**	8	1.99	0.0826	8	1.62	0.1584
Error	46			30			32		

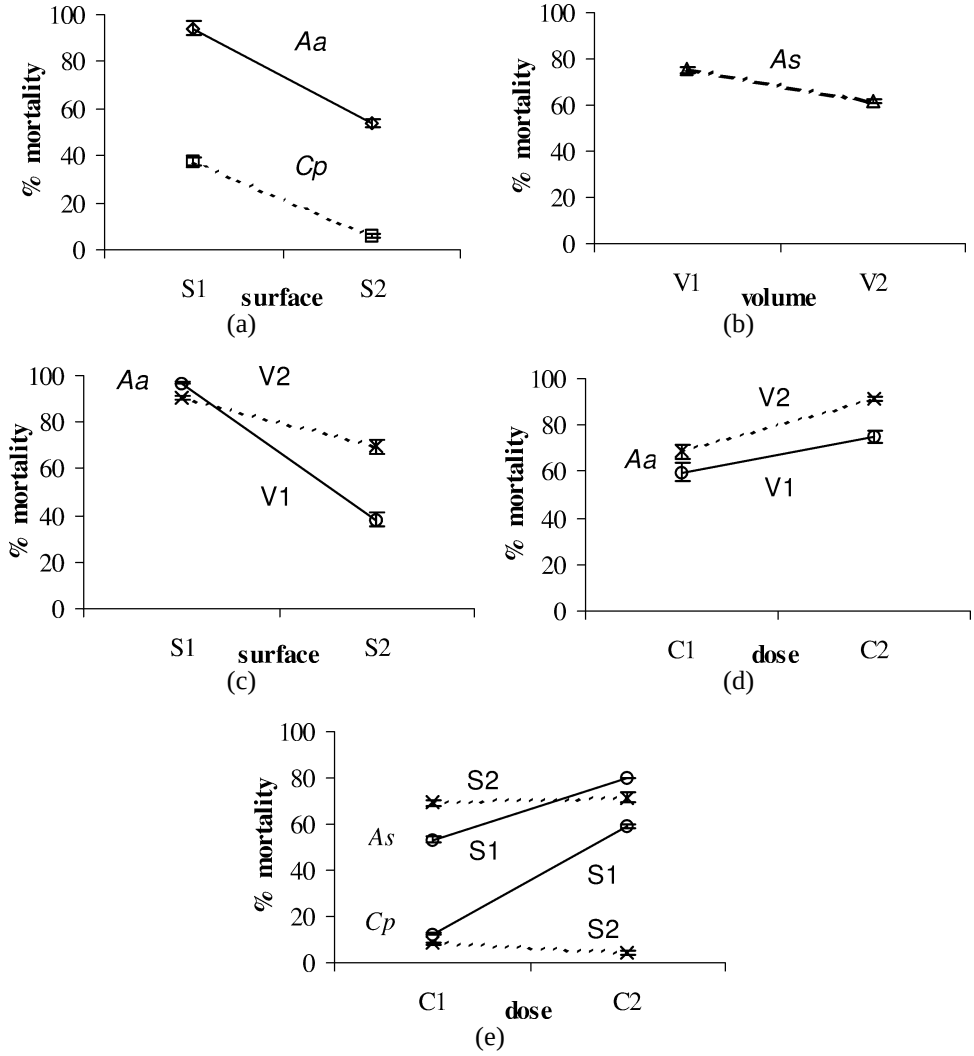

Fig. 1. Significant surface, volume and dose effects on the larval mortality (% ± SE) after treatment with leaf litter of *Ae. albopictus* (*Aa*), *Cx. pipiens* (*Cp*), and *An. stephensi* (*As*). All effects were observed for each larval type at the second time of exposition used for each toxic material (see Table 2). (a) Surface effect after treatment with leaf litter of *Aa* (observed at T_2) and *Cp* (observed at T_4). (b) Volume effect after treatment with leaf litter of *As* (observed at T_2). (c) Surface × volume interaction after treatment with leaf litter of *Aa* (observed at T_2). (d) Dose × volume interaction after treatment with leaf litter of *Aa* (observed at T_2). (e) Dose × surface interaction after treatment with leaf litter of *As* (observed at T_2) and *Cp* (observed at T_4).

All these factors are to be taken into account for examining the real interest of any larvicidal strategy.

4.1. Comparative bioavailability characteristics of leaf litter, Bti *and temephos for mosquito larvae*

The bioavailability for target larvae of a xenobiotic in the ambient water is primarily influenced by its physicochemical performances in the medium [29]. This allows us to distinguish water-soluble materials (e.g., temephos), which tend to be homogeneously distributed throughout the whole medium whatever its geometry, from water-insoluble *Bti* and leaf litter, distributed under more or less stable suspensions. Because of the thinner size of their particles, *Bti* suspensions are more stable in the water column than leaf lit-

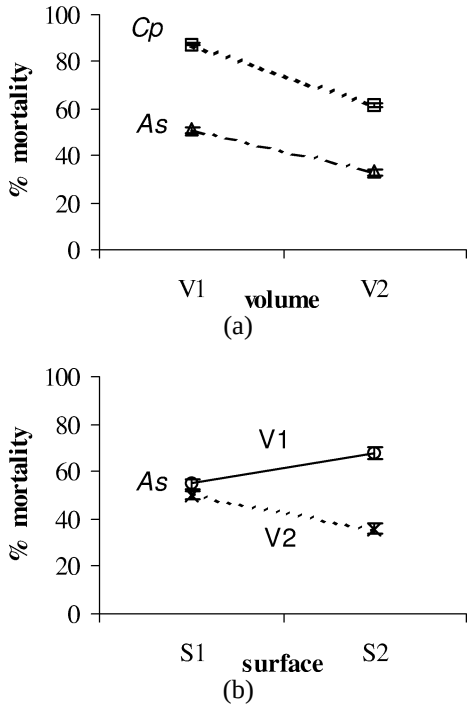

Fig. 2. Significant surface, volume and dose effects on the larval mortality (% ± SE) after treatment with *Bti* of *Ae. albopictus* (*Aa*), *Cx. pipiens* (*Cp*), and *An. stephensi* (*As*). All effects were observed for each larval type at the second time of exposition used for each toxic material (see Table 2). (a) Volume effect after treatment with *Bti* of *As* (observed at T_6) and *Cp* (observed at T_6). (b) Surface × volume interaction after treatment with *Bti* of *As* (observed at T_6).

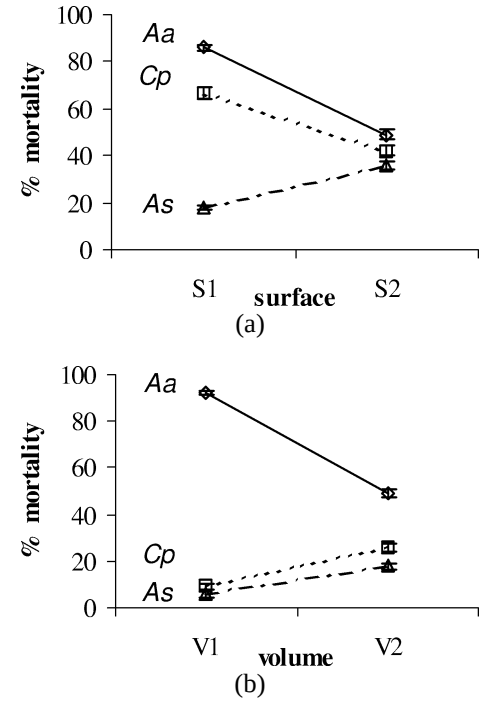

Fig. 3. Significant surface, volume and dose effects on the larval mortality (% ± SE) after treatment with temephos of *Ae. albopictus* (*Aa*), *Cx. pipiens* (*Cp*), and *An. stephensi* (*As*). All effects were observed for each larval type at the second time of exposition used for each toxic material (see Table 2). (a) Surface effect after treatment with temephos of *Aa* (observed at T_6) and *Cp* (observed at T_2) versus *As* (observed at T_6). (b) Volume effect after treatment with temephos of *Aa* (observed at T_6) versus *Cp* (observed at T_2) and *As* (observed at T_6).

ter suspensions [15], which sink according to the basal surface geometry of the water column. Such a variable basal distribution of leaf litter particles may greatly influence their bioavailability for *Ae. albopictus* and *Cx. pipiens*, living much deeply in the water column, rather than for *An. stephensi*, living at the top of the water column [20,30]. In the case of *Ae. albopictus* and *Cx. pipiens*, our modified bioassay results reflect those bioavailability differences between *Bti* and leaf litter according to the environmental surface or volume (i.e., depth) of the water column.

The relative larvicidal performances of leaf litter, *Bti* and temephos are also influenced by the larval behaviour. This appears to be the case for temephos, where differential surface and volume (i.e., depth) effects oppose *An. stephensi*, living at the water surface, from *Cx. pipiens*, and *Ae. albopictus*, living much deeply in the water column. Such an interaction may be of key importance for *Bti* and leaf litter, as the differential larval feeding behaviours appear to greatly interfere with the differential distribution of those dietary larvicidal materials throughout the water column. This is clearly suggested by the surface and volume (i.e., depth) effects discriminating *An. stephensi*, feeding at the water surface, from *Cx. pipiens* and *Ae. albopictus*, feeding in much deeper water or at the bottom [31,32].

Moreover, the differences in larval feeding rates may be crucial in the differential larval tolerance against toxic dietary xenobiotics [33]. This has been observed for Anophelinae, which are less susceptible to *Bti* than other mosquito taxa, because of their low feeding rates [34]. This is also true for leaf litter, where the most sensitive taxa (i.e., Aedinae) are those that can ingest the maximum amount of toxic material

because of their active collecting–gathering feeding mode [35].

4.2. Comparative efficacy of leaf litter and conventional insecticide strategies in larval mosquito control

The results of our modified bioassays suggest that the mortality rates obtained through standard bioassays are to be modulated by the environmental conditions in conjunction with the own larval behaviour. The influence of those abiotic and biotic factors may thus be taken into account to understand the real efficacy of a given larvicide against a given larval type.

Our current results confirm that chemical insecticides (e.g., temephos) are indistinctively effective against widespread anthropophilic larval *Aedes*, *Culex* and *Anopheles* taxa. These wide toxicological performances, however, are modulated at the operational level by environmental and human health concerns [36,37] together with emergence of resistance phenomena in the target fauna [38]. In the same way, the larvicidal use of *Bti* in human dominated breeding sites is checked by its differential efficacy against anthropophilic culicine taxa [19], the presence of environmental factors affecting its bioavailability to target larvae [39], and its ephemeral time of action [40].

Although the standard bioassays indicate larvicidal performances for toxic leaf litter lower than for conventional larvicides, the modified bioassays reveal the bioavailability performances of this phyto-larvicide, which, in return for a sufficient dose in the medium (e.g., 200 mg l^{-1}), displays acute toxicity effects more rapidly than conventional larvicides [15]. Moreover, a possible use of this vegetable larvicide may be recommended in human dominated mosquito breeding sites because of its environmental interest. Interestingly, crude leaf litter material represents *per se* a whole insecticide formulation, i.e., including both the active ingredient and the adjuvant. Whereas the active ingredient is not yet precisely characterized [11,13], a preliminary purification procedure revealed that its toxic efficacy would be 100-fold higher than that of crude leaf litter [12]. The adjuvant corresponds to the non-toxic components of the cell-wall fraction remaining after the ten-month decaying process of the foliar material [7]. This cell-wall material is both stable in the aquatic medium [11] and appetent for the larvae [14]. Such biological performances, associated to the sinking ability of crude leaf litter suspensions in the water column, predispose this material to control preferentially *Aedes* taxa, which feed on the substratum [20]. Innovative control strategies are there needed urgently because of the progressive inefficacy of conventional insecticides which have been over-used against those invasive taxa [41] known for their wide vector competence [42].

5. Conclusion

As shown by the current experimental study, the bioavailability of xenobiotics for mosquito larvae in the aquatic medium appears to result from the complex interaction among several factors: dose and physicochemical characteristics of the xenobiotic, time of exposition, geometry of the water column, and larval behaviour. As those xenobiotics may be of dietary and/or toxicological interest, knowledge of such interactions may allow us to better understand the nutritional and/or toxicological ecology of mosquito larvae, in order to improve control strategies.

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