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Comparative study of the effect of *Bacillus thuringiensis* on larval populations of *Culex pipiens* L. (Diptera-Culicidae) of the City of Tlemcen (Algeria)

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ABSTRACT

In the cities of Algeria, *Culex pipiens* L. (Diptera: Culicidae) is the mosquito which presents most interest because of its wide geographical distribution and of its abundance which engender a strong nuisance. Besides, its role of vector of the virus West Nile arouses a particular interest in the Mediterranean Basin. These insects are generally controlled by conventional insecticides for the greater part, chemicals which cause in the long term side effects (effects on the not aimed bodies and the resistance of the aimed species). A research for the effect of the bioinsecticide *Bacillus thuringiensis* (granulated commercial shape in 200 IUT^1/mg) was realized on préimaginales populations of the artificial deposits sites (taken directly of them natural the deposits sites) and cleansed populations (stemming from a breeding) having never been handled previously, taking into account local weather and physico-chemical conditions. Analyses of variance, allowed to determine the combined effect of the factor measure and of the factor time which by increasing, increase the efficiency of the product. The results of the rates of mortalities registered after treatment allowed to loosen the DL50 and the DL90 for every embryonic stage. From the results, we estimated the degree of sensibility of the larva populations of *Culex pipiens* which have proved heterogeneous in partial tolerance in *Bacillus thuringiensis* for the populations of the sensitive artificial and homogeneous the deposits sites for those stemming from the breeding.

Keywords: Tlemcen; *Culex pipiens*; *Bacillus Thuringiensis israelensis*; Nuisance; Bio Insecticide

1. INTRODUCTION

The situation of nuisance, caused by *Culex pipiens* L. 1758 and strongly felt in most cities in Algeria including the city of Tlemcen [1], interested us to study its ecology, for the sole purpose of establishing a more effective fight.

The ecological plasticity of this species allows it to grow in most deposits and to put up with the wider variations of ecological factors [2-6,11].

The creation of the deposits sites remains the result of the man carelessness. The hardly controlled extension of urban and industrial pollutant sector added to the sewage and sanitation systems are generators of deposits of *Cx. pipiens* [5].

The resistance of these insects to toxic chemical compounds, non-biodegradable, prompts to continually review the ways to fight [7].

Biological control is an alternative and an element of the strategy defined element but difficult to implement.

Discovered during research aimed at developing new biological agents for the fight against tropical disease vectors (example: malaria and onchocerciasis...), *Bacillus thuringiensis israelensis* (*Bti*) showed a significant larvicidal effect on many mosquito species. In 1985, 72 species of mosquitoes were susceptible to the action of *Bti*. Thirteen years later, that number had risen to more than 115 species [8].

In this context, this work aims to estimate and compare the effect of this bio insecticide on *Cx pipiens* larval populations from livestock and those taken directly from the deposits, with toxicology tests performed according

to the method recommended by the WHO[1] [9] in order to demonstrate that the bacteriological and physicochemical conditions may play a role in the effectiveness of *Bti*.

Moreover, if the evaluation of the effect of insecticide treatment on larval populations is usually done in the field, in natural environments, this study analyzes the effects on populations from breeding after purification.

2. MATERIALS AND METHODS

Toxicological tests are conducted in two ways:

- Populations from livestock breeding: the larval hatchlings of hypogean deposits are grouped in lots of 20 larvae at stage 4 placed in cages. Laid eggs are retrieved in 200 ml crystallizers. Adults (30 males and 10 females) are evenly distributed in five different cages. The food, provided daily, of the larvae is composed of a mixture of biscuit and dried yeast (75/25). This is done in May at an ambient temperature of 25˚C and a photoperiod 14/10 hours [10].
- Populations sampled from deposits: the larvae from the four stages are taken directly from three hypogean deposits (crawl spaces) which are located in different neighborhoods of Tlemcen urban group. Water intake is putrid, nauseating and heavily loaded with organic matter from the leakage of defective pipes (rate of conductivity).

The bio insecticide used is the standard commercial formula Vectobac at 200 IUT/mg, granules. The tests were repeated three times on the same larval stage and were performed on the same number of larvae (50 larvae of the same stage), placed in the same volume of water in 1000 ml test tubes, at an ambient temperature between 20 and 25˚C.

The water used is distilled and seven doses decreasing from 35 to 2 mg/L were tested on each stage of larvae from breeding and from 100 to 10 mg/L for those taken from the deposits. For each test a control group (50 larvae of the same stage) was placed.

Dead larvae are removed at regular time intervals (30 minutes) until the death of all larvae (100% mortality).

The results are analyzed by a statistical treatment, developed by the software Minitab12. Mortalities are expressed as mean and standard deviation, calculated on the percentage mortalities of the three tests and corrected [11], this allows to eliminate the natural mortality and to know the actual larvicide toxicity. Variance analyses with controlled factor were used to demonstrate the effect of time, the effect of dose and the effect of larval stage. The regression lines are based on Swaroop & Uemera (1966) method to determine the LD50 and LD90 of the different larval stages, with a probability of 95%.

[1]international toxicity units.
[2]lethal dose for 50% mortality.
[3]World Health Organization.

3. RESULTS

3.1. Determination of the Efficiency of *Bti*

For all doses tested, the bio insecticide treatment causes a significant lengthening of the duration effect ($p < 0.001$). It goes from 330 minutes in high doses to 510 minutes for the dose of 50 mg/L.

The *Bti* time effect is shorter on populations from breeding than on populations taken directly from deposits (**Figures 1** and **2**).

The analysis of variance shows that under the effect of the same doses, the mortality rates recorded 360 minutes after treatment of larvae are high (**Figures 1** and **2**). In the four larval stages ($p < 0.005$), there was a highly significant difference between the average mortality for all instars.

Taking into account the statistical analysis, it is clear that the *Bti* has effects on larval mortality, especially for higher doses, where the larvae are all dead.

The first stage treated larvae, appear to be more sensitive to *Bti*, due to mortality rates recorded for the different concentrations. For larval stages L2, L3 and L4, doses act the same way on the mortality of larvae as well as purified populations of artificial deposits.

The effectiveness of the two factors “dose” and “larval stage” is demonstrated through an analysis of variance with two controlled factors, encompassing the four larval stages and the different doses tested. This analysis shows the impact of each of the two factors; the larval stage and the different doses used, significantly, affect larval mortality of *Cx. pipiens*: probability $p < 0.005$ (**Table 1**).

3.2. Determination of Lethal Dose (Table 2)

The coefficients of the carried out eight regression lines show that there is a significant relationship between the dead larvae and the *Bti* different dosages.

The probit analysis allowed us to retain 50% lethal dose (LD50) of *Bti* which is of the order of 15.87 mg/L for the first larval stage of the breeding population collected from polluted deposits, its upper and lower limits are respectively upper 14.74 and 17.09 mg/L. for this same level of rearing larvae from the LD50 is 2.53 mg/L, its upper and lower limits are 2.26 to 2.83 mg/L.

For larvae of the second stage, the LD50 of larvicide is 20.36 mg/L. Confidence intervals of LD50 are from 12.05 to 34.41 mg/L (larvae from polluted deposits). For larvae from breeding, the LD50 is 3.11 mg/L and confidence intervals are from 2.8 to 3.45 mg/L.

The lethal dose for 50% mortality for larvae stage 3 is 19.44 mg/L. The lower limit is 10.56 mg/L and the upper limit is 35.76 mg/L, this for individuals collected directly from shelters. LD50 populations from livestock is 5.3

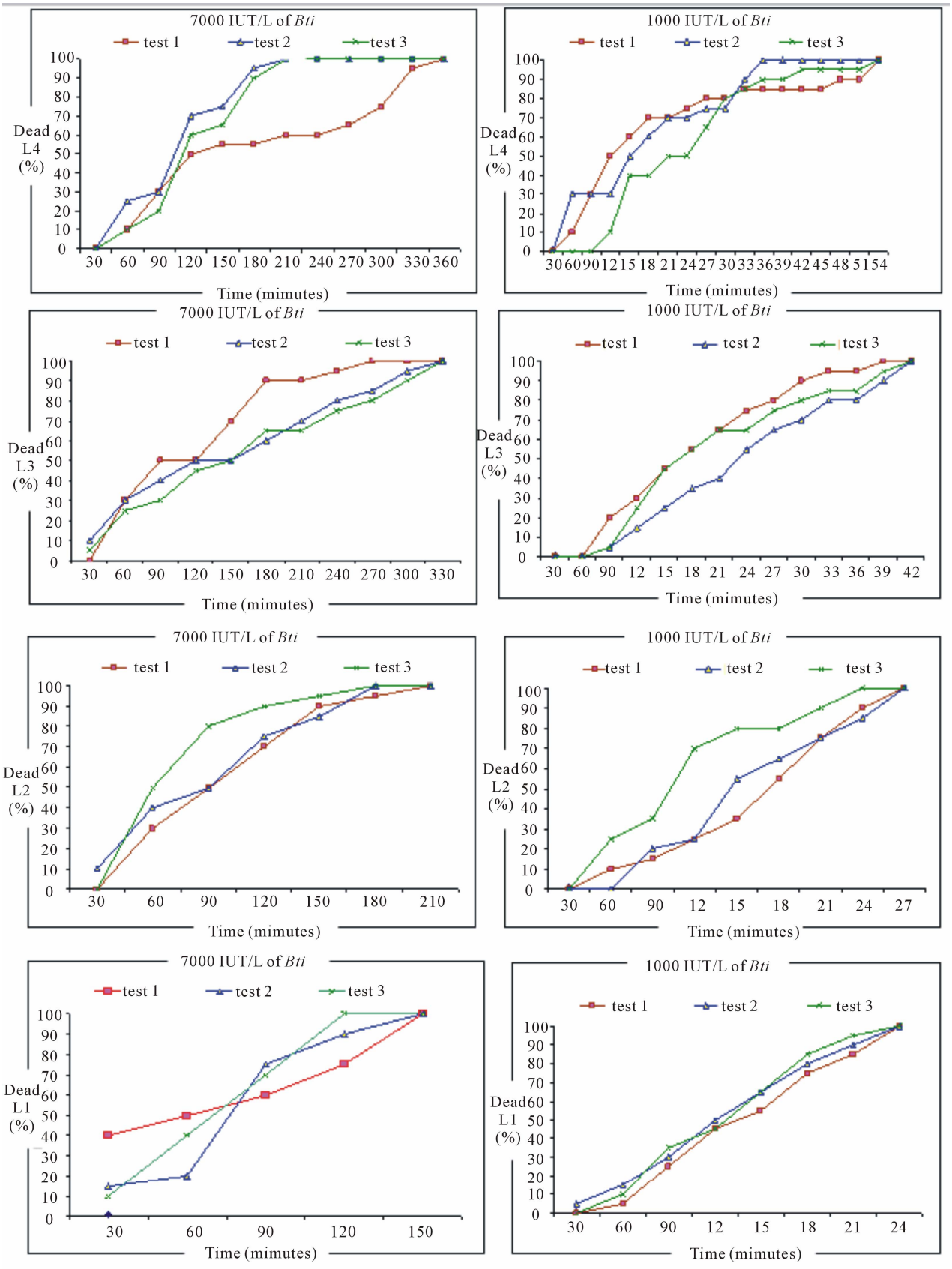
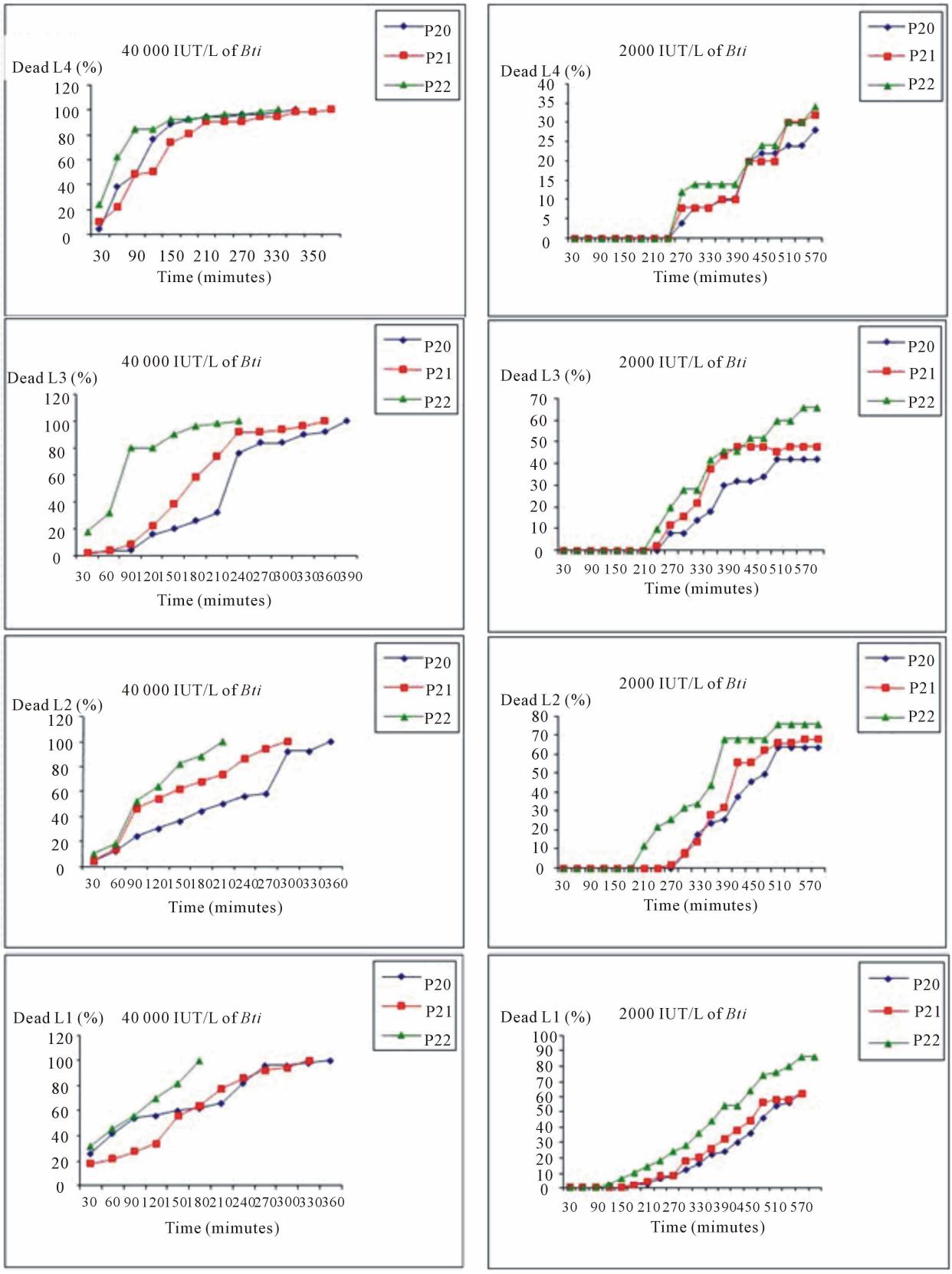


Figure 1. Test Results of *Bti* larvae L4, L3, L2 and L1 of purified populations.



(P20: test 1; P21: test 2; P22: test 3) We began again the same test 3 times for P22: test 3 every larvae stage L4, L3, L2 and L1.

Figure 2. Test Results of Bti on larvae L4, L3, L2 and L1of artificial deposits.

Table 1. Effects of *Bti* on each instar for seven hours (average of three trials followed by standard deviation). (a) Immature populations from breeding; (b) Immature populations taken directly from the field.

(a)

Stade/Dose (mg/L)	1	2	3	4	5	6	7
L1*	60.0 ± 5.0	61.6 ± 5.7	75.0 ± 5.0	86.6 ± 5.7	86.6 ± 5.7	95.0 ± 5.0	100
L2*	58.3 ± 5.7	80 ± 8.6	93.3 ± 5.0	80.0 ± 8.6	95 ± 5.0	98.3 ± 2.9	100
L3*	50.0 ± 5.0	73.3 ± 7.6	80.0 ± 8.6	81.6 ± 2.8	86.6 ± 2.9	95.1 ± 5.0	98.3 ± 2.8
L4*	13.3 ± 2.8	31.6 ± 5.7	41.6 ± 2.9	50.0 ± 5.0	61.6 ± 2.8	73.3 ± 7.6	83.3 ± 2.9
Total*	45.4 ± 3.9	61.6 ± 6.3	72.4 ± 5.3	74.5 ± 4.9	82.4 ± 4.7	90.4 ± 4.5	95.4 ± 0.9

(b)

Stade/Dose (mg/L)	1	2	3	4	5	6	7
L1*	10.6 ± 2.3	28.0 ± 7.2	86.6 ± 5.0	86.6 ± 14.4	75.3 ± 7.5	95.3 ± 4.1	100
L2*	25.3 ± 6.1	65.3 ± 15.5	66.0 ± 2.0	87.3 ± 7.0	88 ± 9.1	93.3 ± 3.0	100
L3*	24.6 ± 11.5	64.0 ± 8.0	63.3 ± 11.3	86.6 ± 11.3	86.6 ± 3.0	86.6 ± 1.1	96.6 ± 4.1
L4*	10.6 ± 1.1	20.6 ± 12.0	32.0 ± 5.2	76.0 ± 5.2	84.0 ± 7.2	84.6 ± 11.0	96.6 ± 4.1
Total*	17.7 ± 4.7	44.4 ± 10.1	61.9 ± 5.3	84.1 ± 8.9	83.4 ± 6.1	89.9 ± 4.2	98.3 ± 1.5

(a) *For each instar, the asterisk indicates a highly significant difference ($P < 0.001$) by ANOVA. (b) *For each instar, the asterisk indicates a highly significant difference ($P < 0.001$) by ANOVA.

Table 2. 50% Lethal doses expressed in mg/L (ITU between brackets) and the fudicial limits.

Larval stage	L1	L2	L3	L4
Purified populations	2.53 (506)	3.11 (622)	5.3 (1060)	9.65 (1930)
Populations sampled from deposits	15.87 (3174)	20.36 (4072)	19.44 (3888)	40.82 (8164)
Proportion	1/6	1/6	1/4	1/4

Larval stage	L1		L2		L3		L4	
Limits	Lower	Higher	Lower	Higher	Lower	Higher	Lower	Higher
Purified populations	1.45	12.93	2.01	16.71	5.64	28.94	16.63	45.62
Populations sampled from deposits	14.74	17.09	12.05	34.41	10.56	35.76	26.5	62.87

mg/L, its upper and lower limits are 4.18 to 5.83 mg/L. The results of the fourth larval stage are: for larvae collected lodges, the LD50 was 40.82 mg/L, its limits are 26.50 to 62.87 mg/L. for larvae from breeding, the LD50 is 9.65 mg/L and confidence intervals are 8.93 to 10.42 mg/L.

Lethal doses LD50 and L90 are relatively lower for breeding populations. It should be noted that only 1/5th of doses for the same mortality between larvae reared in clean water and those taken from the deposits. This difference is significant for all doses and all larval stages.

According to sensitivity tests [12], préimaginales populations of Cx. pipiens polluted lodges are heterogeneous populations, a party may be highly sensitive and one tolerant. Larvae from breeding proved consistent and sensitive to *Bti* (**Table 3**).

4. DISCUSSION

Time, dose and larval stages effects are functional in the same way for the two populations of studied *Culex pipiens*.

Factors such as environmental parameters (larval density, water temperature...) can significantly affect the effectiveness of *Bti*.

Mosquito species show different levels of susceptibility to *Bti crystals*. In general, *Culex* larvae are the most sensitive, and the larvae of *Aedes* and *Ochlerotatus* are equal or slightly less sensitive and *Anopheles* larvae are more resistant when exposed to the same amount of *Bti* crystals. This difference in susceptibility within the same genus (e.g. species belonging to the genera *Culex*, *Aedes*, *Anopheles* or *Ochlerotatus*) is caused by behavioral [13] and physiological changes of the various species, but it is clearly linked to the behavior of crystals in the environment [14-16].

Although a difference in the type and a number of "receptors" may exist between the various mosquito species [17], the same number of *Bti* crystals induce a lower mortality rate in cold water than in hot water [17,18]. This toxicity decrease is due to a reduction of metabolic activity (reduction of ingestion and enzymatic activity) observed when insect is exposed to temperatures approaching the minimum temperature at which it is normally found in the environment. It should be noted that at low temperatures, some formulations show a low rate of mixing and dispersion, which reduces the availability of *Bti* crystals.

Generally, in most of studied species, the youngest larvae are more susceptible than the older [19]. Ageing, the larvae become significantly less sensitive to the

Table 3. Results of sensitivity tests to *Bti* on the four Cx. pipiens instars. (a) Immature populations sampled in the belowground deposits; (b) Immature people from breeding.

(a)

Stage	LD50	LD90	K = corrected LD50/LD50 basis	P = corrected LD90/corrected LD50	Interpretation
Stage 1	15.87	48.35	0.86	3.04	Heterogeneous partial tolerance
Stage 2	20.36	55.84	1.11	2.74	Heterogeneous partial tolerance
Stage 3	19.44	92	1.06	4.73	Heterogeneous partial tolerance
Stage 4	40.82	133	2.23	3.25	Heterogeneous partial tolerance

(b)

Stage	LD50	LD90	K = corrected LD50/LD50 basis	P = corrected LD90/corrected LD50	Interpretation
Stage 1	2.53	6.16	0.14	2.43	Sensitive homogeneous
Stage 2	3.11	7.65	0.17	2.45	Sensitive homogeneous
Stage 3	5.30	13.15	0.29	2.48	Sensitive homogeneous
Stage 4	9.65	21.25	0.52	2.20	Sensitive homogeneous

same number of *Bti* crystals. In general, stage 2 larvae are 1.5 to 5 times more sensitive than stage 4 larvae [15, 18]. Stage 4 larvae feed very little, as they start pupation [19].

Generally, the more the settlement contains organic matter and colloidal matters in suspension, the more the number of *Bti* crystals must be high for the same mortality rate [20,21]. The adsorption of the crystals on the particles, followed by a slow precipitation, reduces the availability of *Bti* crystals. In addition, larvae exposed to high concentrations of "nutritious" particles can prove reduced ingestion rates, suggesting that they have reached the level of satiety [15]; so the larvae will ingest less crystals causing a decrease in mortality. The presence of organic pollution also reduces the toxic activity [17].

According to the sensitivity test, the larvae in contaminated deposits proved to be heterogeneous with partial tolerance. The product does not take effect on a regular basis on all individuals within a population and even within a single generation. Some died in the first two hours and some others, however, others took a much longer time up to 16 hours. For the more advanced stages, L3 and L4, we noted that approximately 3% of the individuals tested are highly tolerant. Thus, there is a time effect that can be explained by bacterial spores that proliferate gradually in epithelial cells.

Larvae from livestock are homogeneous and susceptible, these individuals reared in clean water would have a less effective immune system than the larvae grown in contaminated water rich in microorganisms.

Preimaginal populations, living in wastewater highly loaded in bacteria in particular, require lethal doses at 50 and 90%, significantly higher than those from livestock. These larvae thus have a certain tolerance towards *Bti*. The water pollution would be responsible for the decrease of the effect of *Bti* crystals [17,22-24].

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