Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Annals of Biological Research, 2013, 4 (11):85-92
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

Effect of *Bacillus thuringiensis* var *israelensis* against *Culex pipiens* (insecta: Culicidae). Effect of *Bti* on two non-target species *Eylais hamata* (Acari: Hydrachnidia) and *Physa marmorata* (Gastropoda: physidae) and Dosage of their GST biomarker

M. Mansouri*, F. Bendali-Saoudi*, D. Benhamed* and N. Soltani*

Laboratory of Applied Animal Biology Department of Biology, Faculty of Sciences, Badji Mokhtar University of Annaba, Annaba (Algeria)

ABSRACT

The present work aims to study the effects of the biocide Bacillus thuringiensis var israelinsis, against non-target adults of water mites Eylais hamata Koenike, 1897, as well as its associated host species Physa marmorata Fitzinger, 1833. After 12 days of oral treatment of adults with lethal concentration (LC50:0.08µg/ml), determined from essays on 4th instar larvae of Culex pipiens (hematophagous insects). No adverse effect has been recorded for adult individuals of Eylais hamata, contrary, snail Physa marmorata were sensitive for this dose of Bti. In parallel, after treatment at the Bti by LC50, the enzyme stress biomarker glutathione S-transferase, was measured after 24, 48 and 72 hours. The enzymatic activity of GST has increased following treatment.

Key words: Biocide, *Bacillus thuringiensis* var *israelinsis*, biomarker, enzymatic activity.

INTRODUCTION

Chemical control continues to be the major means of control despite its adverse consequences on the environment; inter alia, by toxicity in the food chain, the pollution of surface water and groundwater [1]; [2]; [3]; [4]; [5], the appearance of resistant strains [6]; [7], due to the untimely, unconditional and irrational chemical pesticide use [8]; [9]; [10]; [11].

The achievement at the level of non-target species was reported following the impulsive use of chemicals affecting the viability and reproduction of non-target fauna. The water mites (Acari: Hydrachnidia) constitute an important group in ecological monitoring, these excellent indicators of the quality of the habitat have radiated from the Triassic to over 5000 described species, occupying almost all freshwater [12], and parasites for many species. Also, the freshwater gastropods are host species of the water mites [13], thereby, their presence in the same environment is important.

Because of their predatory potential for larvae of Culicidae [14]; [15]; [16],water mites are an important means of biological control against this harmful species. The Culicidae include many species that were identified among the most important hematophagous ectoparasites. However, they are vectors of diseases and viruses [17], as plasmodium [18], Malaria [19] and the West Nile virus mainly transmitted by the *Culex* genus [20]; [21].

To combat these pathogens, and to preserve the non-target fauna, biological control is an effective alternative in natural environments, because it offers viable solutions for his activity of entomopathogenic and phytopathogenic microorganisms because of their specificity for target organisms, their intrinsic compatibility with the natural environment and their evolutionary action with and without human intervention. At the present time, *Bacillus*

thuringiensis var *israelinsis* is among the most commonly species used in fight against harmful insects. *Bti* is used for the selective control of larval populations of the mosquito diseases vectors in many types of continental aquatic ecosystems [22]; [23], as well as black flies [24]; [25]; [26], and species of Diptera [27].

Bti is known for its biodegradation and its specificity for non-target species as well as its mode of action which is specific. When *Bti* proteins are ingested by larvae of mosquito, the parasporal body dissolves in the alkaline juices of the midgut activating protoxin to release active proteins of delta-endotoxin [28], because, the endotoxins are originally bound in stable molecules of protoxin [29]; [30]. Once linked to specific receptors present on the membranes of midgut epithelial cells, the toxin induces the formation of pores in the membrane of epithelial cells and causing the death of cells and infected larva [31]; [32]; [33]; [34].

The glutathione S-transferase (EC: 2.5.1.18) have a role in the detoxification of xenobiotics substances or endogenous in catalyzing the conjugation of these substances with the endogenous glutathione thiol group [35]; [36]; [37]; [38]. These class GSTs contribute to the phase II conjugation securing endogenous hydrophilic derivatives on the functional groups of the phase I. These enzymes are used as a biomarker for the early detection of the presence of the present xenobiotics in the environment.

The aims of this work is to demonstrate the toxic effect of the *Bti* on *Culex pipiens* as well as to determine the effect by oral application of the *Bti* on adults of *Eylais hamata* and its associated species *Physa marmorata,* in order to show its effect on non-target populations of water mites and of freshwater gastropods. We studied also the metabolic effects after treatment with LC50 of *Bti* as a response to exposure to the biocide of both species of *Eylais hamata* and *Physa marmorata*. This is by measuring the enzymatic activity of glutathione S-transferase, biomarker of environmental stress.

MATERIALS AND METHODS

Sampling sites

Culex pipiens mosquitoes were collected in the region of Sidi Ammar, suburban zone in the Wilaya of Annaba, located in the North-East of Algeria. Species of Eylais hamata were sampled from the Lake of Beards (Lac des Oiseaux) in Wilaya of El Taref, extreme Algerian North-East. The presence of water mites were observed at the level of the stations whose depth is extremely minimal, in association with the snails, *Physa marmorata*.

Biological material

Culex pipiens belongs to the Culicidae family, which includes mosquitoes which are Diptera Nematocera, female is temporary hematophagous ectoparasites, its life cycle involves two phases, an aquatic larval development (4 larval and nymphal stages), and one aerial phase with sexual dimorphism. Culicidae represent nuisance in our study area, and main vector of West Nile virus in the world. The hydrachnidae, order of the Acari, include the water mites that are little studied throughout the world. *Eylais hamata* is the most abundant species in the Lake of birds. Water mites larvae are parasites for many species in fresh water. *Physa marmorata* gastropod mollusc is living in stagnant or low current fresh water, and feeds on algae. It can become invasive in favorable conditions.

Farming

Adults of Culex pipiens were kept in cages until laying. Eggs have been maintained in the laboratory in plastic boxes filled with dechlorinated water, and larvae are fed with algae of fish, under a temperature between 30 - 35 C ° and a photoperiod of 12 h until the fourth instar larvae. The individuals of *Eylais hamata* and *Physa marmorata* have been maintained in the laboratory for stabling, in the boxes made of plastic filled with dechloruree water, at an ambient temperature of 25-27 ° C.

Insecticide

The insecticide used is the biocide *Bacillus thiringiensis* var *israelensis* in its commercial form Vectobac WDG 37.4% active ingredient.

Toxicity essay

The toxicity essays were carried in round plastic boxes 10 cm in diameter x 7cm in height and filled with 200ml of dechlorinated water. Colonies *of Culex pipiens* L4 were treated separately by the *Bti* with different concentrations, 0.04µg/ml, 0.06µg/ml, 0.11µg/ml, 0.22µg/ml. Three replicates were used, for each 20 individuals have been tested with the controls.

Le *Bti* has been prepared in distilled water, and the LC50: 0.08 µg/ml was applied on non target species, *Eylais hamata* and *Physa marmorata*. For each dose, 3 repetitions and 3 controls has been carried. Each test contains 20 species.

GlutathioneS-transferase essay

The LC50 was applied orally to individuals of *Eylais hamata* and *Physa marmorata*, *Bti* was dosed at his LC50. Then, samples were collected at 24, 48 and 72 h after treatment. For GST activity measurements, we used 4 pooled bodies per repeat and 4-5 repeats per time interval. The assay of GST was carried out according to [39] with use of GSH (5 mM) and 1-chloro-2-4-dinitrobenzoic acid (CDNB, 1 mM). Adult decapitated bodies of water mites and shelled gastropods were individually homogenized in 1 ml of buffer phosphate (0.1 M, pH 6). The homogenate was centrifuged (1400 rpm for 30 min) and the supernatant collected used for the enzymatic assay. After, 200 µl of the resulting supernatant were added to 1.2 ml of the mixture GSH-CDNB in phosphate buffer (0.1 M, pH 7). Changes in absorbance were measured at 340 nm every minute for a period of 5 min.

Statistical analysis

The mortality percentages were corrected for control mortality (<20%) with Abbott's formula. We used the software Graphpad Prism5 to calculate the lethal doses (LD50 and LD90) with their corresponding 95% fiducial limits (95%FL).

Results are presented as means ± standard deviation (s.d). The significance between different series was tested using Student's t-test at 5% level. Statistical analyses were performed using MINITAB software V16 and $p < 0.05$ was considered statistically different.

RESULTS

Bti activity against Culex pipiens

The 4th Instar larvae of *Culex pipiens* treated with *Bti* showed low activity followed by death and darkening of species. The percentage of mortality after treatment was determined according to different doses of *Bti* (0.04µg/ml, 0.06µg/ml, 0.11µg/ml and 0.22µg/ml) and the time oftreatment (24, 48 and 72 h). After treatment with highest dose of *Bti* (0.22µg/ml), the corrected mortality increased to 95.83, 98.61 and 100 after 24, 48 and 72h respectively.

Table 1. Lethal doses of *Bti* by oral application on of 4th instar larvae of *Culex pipiens*. The data are expressed as lethal doses LD50and LD90 (µg/ml) together with the corresponding 95% fiducial limits (95%FL) as function of the exposure time (hours)

Time (hours)	Slope	LC_{50} 95% FL (µl)	LC_{90} 95% FL (µl)
24	0.53 (0.84-5.46)	355.1 (276.3-456.4)	712.7 (392.5-1294)
48	0.32 (1.96-4.73)	295.8 (260-336.5)	569.9 (417.4-777.9)
72	0.26 (1.69-3.93)	270.4 (236.7-308.9)	590 (420.3-828.2)

Effect of Bti on Eylais hamata

We observed mortalities in *Eylais hamata* adults treated with *Bti* and for controls. The percentages of mortality, were determined based on LC50 (0.08µg/ml) and the time of treatment (days 3, 6, 9, 12); (Fig.1). The mortality rate increased from 5% to 50% and 5% to 45% in the treated adults and controls respectively for 9 days. Statistical analysis indicates that *Bti* is not toxic with a non significant effect ($p > 0.05$) during the 6th and 9th days.

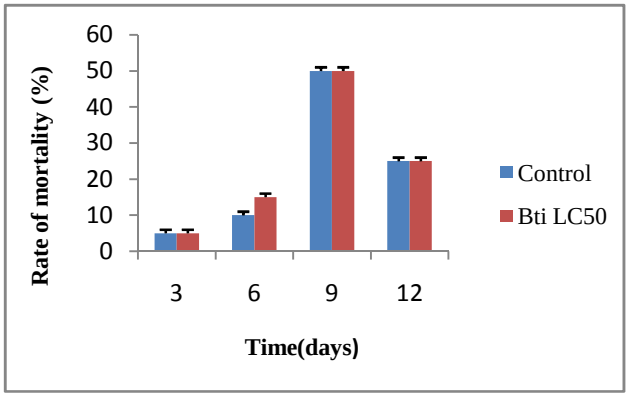

Fig.1: Effect of LC50 of *Bti* on *Eylais hamata*

Effect of Bti on Physa marmorata

We observed mortalities in *Physa marmorata* treated with *Bti* and for controls. The percentages of mortality, were determined based on LC50 (0.08µg/ml) and the time of treatment (days 3, 6, 9, 12); (Fig.2).The mortality rate increased from 1.65% to 16.65% after 12 days of treatments. In controls, the mortality rate increased from 1.65% to 10% during the 9th day. Statistical analysis indicates that *Bti* is toxic with a significant effect ($p < 0.05$) after 12 days of treatment.

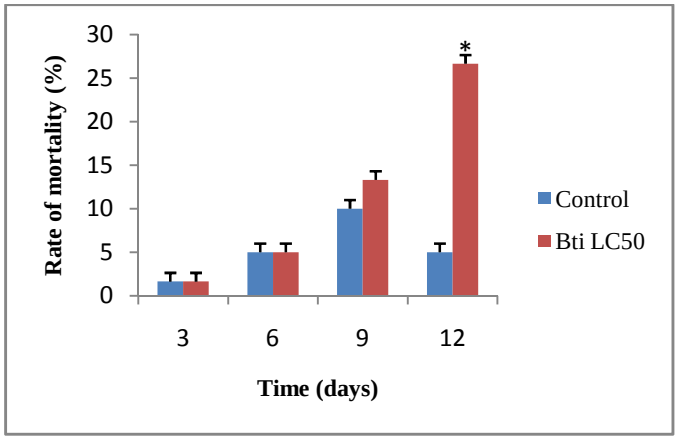

Fig.2: Effect of LC50 of *Bti* on *Physa marmorata*

Effect of Bti on specific activity of GST in non target species Eylais hamata and Physa marmorata

The *Bti* was administered by oral application in adults of *Eylais hamata* and *Physa marmorata.* This biocidal effects were assessed at different times (24, 48, 72 h), on the specific activity of the GST. The results were expressed by contributions to the amount of protein (mg) obtained from a datum curve.

The specific activity of GST was assessed using the slopes of the regression representatives the absorbances lines based on time in controls series.

Effect of Bti on specific activity of GST in Eylais hamata

The results obtained show that the activity of GST (µM/min/mg protein) increases not significantly ($p > 0.05$) after 24, 48 and 72 h the controls of *Eylais hamata*. Among the series treated by *Bti* (LC50: 0.08µg/ml), the results show that the activity of GST (µM/min/mg protein) is not significant after 24 h, it increases very highly significant ($p < 0.001$) after 48 h and significant ($p < 0.05$) after 72 h.

Table 2: Effect of *Bti* (LC50: 0.08µg/ml), on specific activity of glutathion S-transférase (µM/min/mg de protéines) in *Eylais hamata* (m±sd): Comparison of the averages at different times for the same series (lowercase letter) and the same time between the different series (uppercase letters)

Time (hours)	Control	*Bti* (CL50)
24	0.084±0.029 A a	0.103±0.007 A a
48	0.091±0.020 A a	0.258±0.023 B b
72	0.121±0.018 A a	0.539±0.123 B c

Effect of Bti on specific activity of GST in Physa marmorata

The results show that the activity of GST (µM/min/mg protein) increases not significantly ($p > 0.05$) after 24, 48 h. In contrast, after 72 h there is a highly significant ($p = 0.01$) the controls of *Physa marmorata*. Among the series treated by *Bti* (LC50: 0.08µg/ml), the results show that the activity of GST (µM/min/mg protein) is not significant after 24 h. contrary, after 48 h, we observed a significant increase ($p<0.05$), but no significant difference after 72 h ($p > 0.05$).

Table 3: Effect of *Bti* (LC50: 0.08µg/ml), on specific activity of glutathion S-transférase (µM/min/mg de protéines) in *Physa marmorata* (m±sd): Comparison of the averages at different times for the same series (lowercase letter) and the same time between the different series (uppercase letters)

Time (hours)	control	*Bti* (CL50)
24	0.080± 0.018 A a	0.122±0.013 B a
48	0.095±0.01 A a	0.235±0.013 B b
72	0.129±0.008 A b	0.310±0.122 A c

DISCUSSION

Insecticidal toxicity of Bti against Culex pipiens

The transmission of diseases by the Culicidae remains an alarming phenomenon. *Bacillus thuringiensis* var *israelensis* has been used in large scale due to its specificity for Culicidae. Our results show a toxic effect of *Bti* against the 4th instar larvae of *Culex pipiens.* Thus, laboratory and field studies show that *Bti* has an effect against target species: Nematocerae, Culicidae, Simuliidae ; Chironomidae [40], [41]; [42]; [43]; [44]; [45]; [46]; [47]. His larvicidal potential has been demonstrated on various harmful species: larvae of *Culex pipiens* L., *Aedes geniculatus* [48], *Aedes aegypti* [49]; [50], *Anopheles sergentil* [49], *Anopheles stephensi* [50], *Culex pipiens*, *Culex univittatus* and *Uranotaenia unguiculata* [49]. In other studies, [51] observed a reduction in the abundance of mosquito larvae after *Bti* application in different sites.

The *Bti* which specifically affects the Culicidae, contains spores and parasporal crystals of the serotype of *Bti* H – 14, that must be ingested by the larvae of the mosquito to cause mortality. After ingestion, the parasporal crystals are solubilized in the larval alkaline midgut, followed by proteolytic activation of proteins into soluble crystals.The toxin binds to a receptor cells of the midgut wall to form pores in cell, leading to larval death [52]; [53].

The specificity of the *Bti* reduces the possibility that the bacteria survive outside the aquatic environment. Studies on the growth of *Bti* outside the favorable environment of the insect show a very low multiplication outside the host [54].

Effect of Bti on non target species Eylais hamata and Physa marmorata

Bti showed a weak effect on water mites, and this is in accordance with the results of the investigations of laboratory studies, that confirmed the safety of the *Bti* in the presence of non-target species [55]; [56]. Thus, the results of research on the density of aquatic invertebrates: Mollusca, Oligochaeta, Crustacae, Hirudinea, Heteroptera, Ephemeroptera, Odonata, Trichoptera, Coleoptera in different sites in Druskininkai in Lithuania, showed no significant difference [56].

[57] showed no adverse effects of the treatments on the abundance of Polychaeta *Nereis diversicolor* and *Corophium volutator* by *Bti* applications. According to [58], freshwater Cnidaria of the genus Hydra were not affected by *Bti* in laboratory tests at a concentration of 100 mg/L of *Bti*. In addition, [58] showed that oligochaetes of the genus Tubifex were not affected By *Bti* in laboratory tests at a concentration of 180 mg/L. In their 6-year of survey observation on the effects of *Bti* on non-target invertebrates in Minnesota wetlands, [59] did not find any significant difference in the abundance of annelids (including oligochaetes) between control and VectoBac® G-treated areas. Similarly, [60] found no effect of VectoBac® 12AS on the abundance of insect larvae crustaceans or molluscs collected in sediment from temperate New South Wales saltmarshes. Other *in situ* studies, found no significant *Bti* toxicity to chironomids [61]; [62]; [63]; [64]; [65]. In other studies, [51] showed an increase in the taxonomic richness and abundance of protozoans, after *Bti* application *in situ*. After application of the *Bti* by spreading air against mosquitoes of the Dalälven River in the Sweden, there was a production of new insects [63]. Laboratory and field tests showed that *Bti* can be considered as ineffective and harmless biocide to the environment because of its selectivity [66]; [60]; [67]. The World Health Organization declared: "*Bti* is safe for use in aquatic environments including drinking water reservoirs for the control of mosquitoes, black flies and harmful insect larvae" [68].

Other indications show that non-target organisms in wetland can be affected by larvicides containing *Bti* [59]; [69]; [45]. That is according with our results concerning the gastropods *Physa marmorata*, whose were affected by *Bti* after treatment. The effect can be due from the fact that *Bti* has a detrimental effect on the digestive system that can be attributed to the activity of the toxin produced by the bacterium [70]. Also, [71] have shown that *Bti* have a highly suppressive effect on the population growth of *Biomphalaria alexandrina* snails. Moreover, [70] showed that *B. thuringiensis kurstaki* (Dipel-2x) has a potent effect on the survivor and egg laying capacity of Lymnaea

natalensis snails. *Bacillus thuringiensis kurstaki*, was as toxic to snails *Biomphalaria alexandrina* [72]. In addition, [73] showed that *Btk* (Dipel-2x) induced a 50% mortality (LC50) of *Physa acuta*, after 24 h exposure to 270 mg / L. Therefore, *Bti* can affect non-target organisms in different ecosystems such as stagnant water [74] or lotic freshwater [75]; [76].

Effect on biomarker GST

The tolerance of the insecticides in some species represents an ability of the population to resist towards the introduction of exogenous and sometimes polluting products into the environment. The introduction of these substances promotes the induction of metabolic processes of detoxification, resulting in the increase of the activity of certain enzymes including the GSTs [77]; [78]; [79]; [80]. GST enzyme is known as an antioxidant defense mechanism [81]. The specific activity of the GST in adults of *Eylais hamata* as well as *Physa marmorata* was measured after treatment with *Bti*. The increase in GST activity after treatment with *Bti*, reflects an established system of detoxification which, is a form of defense of the organisms against the insecticide [82]. Similarly, increase of GST activity was found in *Aedes rusticus* after treatment with *Bti* [83]. It was demonstrated that viral infection induces GST increasing in mosquitoes [84]. Application of *Bt* to larvae of *Galleria mollonella*, another species of Lepidoptera, induces increase of GST activity [85]. The activity of GST was also increased in mites after treatment by the Pyrethroid [86]. There has been an increase in GST activity in mites *Sarcoptes scabiei* var. *hominis* treated with acaricides, Permethrin et Ivermectin [87]. The evaluation of the enzyme response following the introduction of exogenous products indicates the enzyme functioning in different susceptible species. Therefore, our results are consistent with previous surveys, indicating that the bacterial infection increases susceptibility in arthropods to insecticides and other exogenous toxins, [88]; [89]; [90].

CONCLUSION

Bti toxicity test was conducted on *Culex pipiens*, the LC50 was applied on non-target species: *Eylais hamata* et *Physa marmorata*. *Bti* has shown a toxic effect on the Culicidae. However, the *Bti* affect differently the non-target species. *Bti* induced enzyme stress in the treated organisms, by the increase in GST activity, which can be explained by the establishment of the system of detoxification.

Acknowledgements

This study was financed by the Algerian Fund for Scientific Research and by the Ministry of High Education and Scientific Research of Algeria (CNEPRU and PNR projects to Dr. F. Bendali).

REFERENCES

[1] R A Rudd, **1974**. Pesticides and the Living Landscape. Madison, USA: University of Wisconsin Press.
[2] K Mellanby, **1967**. Pesticides and Pollution. London: Collins, pp.367.
[3] T Roberts, **1989**. Pesticides in drinking water. Shell Agriculture 3, 18-20.
[4] T E Tooby, **1989**. Pesticide contamination of water- regulatory consideration. Proceedings of the Brighton Crop Protection Conference on Weeds 3: 1165-1171.
[5] P Vereijken, P Viaux, **1990**. Vers une agriculture intégrée. Supplément de La Recherche 227: 22-25.
[6] S M Valles, K Dong, R J Brenner, **2000.** *Pestic. Biochem. Physiol* 66: 195-205.
[7] M Fulton, P B Key, **2001.** *Environ. Toxicol. Chem* 20: 37-45.
[8] G P Georghiou, C Mellon, **1983**. Pest resistance in time and space 1-41p.
[9] A L Knight, G W Norton, **1989**. *Annu. Rev. Entomol* 34: 293-313.
[10] M Poiré, N Pasteur, **1991**. La résistance des insectes aux insecticides. La Recherche 22: 874-882.
[11] R L Starnes, C L Liu, P G Marone, **1993.** *Amer. Entomol* 39:83-91.
[12] I M Smith, D R Cook, B P Smith, **2001**. Water mites (Hydrachnida) and other arachnids. *In* Ecology and Classification of North American Freshwater Invertebrates, Chapter 16, pp 551-659. 2nd edition, edited by J H Thorp and A P *Covich. Academic Press* pp 1056.
[13] T Imamura, **1958**. *Jour. Fac. Sci., Hokkaid Univ., Ser.VI, Zool. JI.*
[14] C A Lanciani, A D Boyt, **1977**. *Journal of Medical Entomology* 14:10-15
[15] B P Smith, **1983**. *Agric. Exp. Stn. Univ. Calif., USA* pp 79-85.
[16] B P Smith, S B McIver, **1984.** *Can J. Zool* 62: 1114-1120.
[17] M L Tantely, J C Rakotoniaina, E Tata, L Andrianaivolambo , F Razafindrasata, D Fontenille, N Elissa , **2013**. *Journal of Medical Entomology* 50: 603-610.
[18] C Paupy, B Makanga, B Ollomo, N Rahola, P Durand, J Magnus, E Willaume, F Renaud, D Fontenille, F Prugnolle, **2013**. *PLoS One* 8: e57294.
[19] A M Adja, E K N'Goran, B G Koudou, I Dia, P Kengne, D Fontenille, F Chandre, **2011**. *Annals of Tropical Medicine and Parasitology* 105: 13-24.

[20] G L'Ambert, J B Ferre, F Schaffner, D Fontenille, **2012.** *Journal of Vector Ecology* 37: 269-275.
[21] T M Balenghien, M Vazeille, F Grandadam, H Schaffner, P Zeller, P Reiter, F Sabatier, F Fouque, D J Bicout, **2008.** *Vector Borne Zoonot. Dis* 8: 589-595.
[22] N Becker, **1997**. *Parasitol. Today* 13, 485–487.
[23] N Becker, D Petric, M Zgomba, C Boase, C Dahl, J Lan, A Kaiser, **2003**. Mosquitoes and their Control. *Kluwer Academic/Plenum Publishers, New-York. Boisvert*, J.
[24] J Margalit, D Dean, **1985.** *J. Am. Mosq. Control Assoc* 1:1-7.
[25] D P Molloy, **1990.** Progress in the biological control of black flies with *Bacillus thuringiensis israelensis*, with emphasis on temperate climates, in *Bacterial control of mosquitoes and black flies: Biochemistry, genetics and applications of Bacillus thuringiensis israelensis and Bacillus sphaericus* (de Barjac, H., and D.J. Sutherland Eds), *Rutgers University Press, New Brunswick, New Jersey* pp: 161-186.
[26] J MacFarlane, **1992.** The effects of *Bacillus thuringiensis* var. *israelensis* on non target organisms in the aquatic ecosystem: a literature review. Department of the Environment, Fish and Wildlife Branch, Charlottetown, Prince Edward Island, Canada.
[27] A Ali, **1981**. *Journal of Invertebrate Pathology*. vol. 38: 264–272p.
[28] C N Chilcott, J Kalmakoff, J S Pillai, **1983.** *FEMS Microbiol. Lett* 18:37-41.
[29] I Larget, H de Barjac, **1981**. *B. Soc. Pathol. Exot* 74:216-227.
[30] J F Charles, H de Barjac, **1982.** *Ann. Inst. Pasteur Mic* 133:425-442.
[31] Aronson A.I., Beckman, W., and Dunn, P. **1986**. *Microbiol. Rev* 50: 1-24.
[32] H Höfte, H R Whiteley, **1989.** *Microbiol. Rev* 53: 242-255.
[33] D Lereclus, C Bougouin, M M Lecadet, A Klier, G Rapoport, **1989**. Role, structure, and molecular organization of the genes coding for the parasporal delta-endotoxins of *Bacillus thuringiensis*. In: Regulation of Procaryotic Development (Edited by I. Smith, R.A. Slepecky and P. Setlow). American Society for Microbiology, Washington, D.C pp: 255-276.
[34] M J Adang, **1991**. *Bacillus thuringiensis* insecticidal crystal proteins: gene structure, action, and utilization. In: Biotechnology for biological control of pests and vectors (Edited by K. Maramorosch). CRC Press, London pp: 3-23.
[35] W B Jacoby, W H Habig, **1980**. Glutathione transferase. In: Jacoby W.B (Eds). Enzymatic basis of detoxication (vol2). *Academic press. New York* 63-94.
[36] S Ahmad, **1995**. *Arch. Insect Biochem. Physiol* 29: 135–157.
[37] B Halliwell, J M C Gutteridge, **1999**. *Free radicals in biology and medicine*. Oxford University Press, New York.
[38] S N Radyuk, R S Sohal, W C Orr, **2003.** *Biochem. J* **371**: 743–752.
[39] W H Habig, M J Pabst, W B Jacoby, **1974.** *J. Biol. Chem* 249(22): 7130-7139.
[40] R Garcia, B Des Rochers W Tozer, **1981**. Studies on the toxicity of *Bacillus thuringiensis* var. *Israelensis* against organisms found in association with mosquito larvae. Proceedings and Papers of the Californian Mosquito and Vector Control Association 48: 33-36.
[41] R W Merritt, E D Walker, M A Wilzbach, K W Cummins, W T Morgan, **1989.** *Journal of the American Mosquito Control Association* 5: 397-415.
[42] S Kondo, M Ohba, T Ishii, **1992.** *Lett. Appl. Microbiol* 15: 207–209.
[43] K Liber, K L Schmude, D M Rau, **1998.** *Ecotoxicology*. Vol. 7: 343–354p.
[44] D Rey, A Long, M P Pautou, J C Meyran, **1998.** *Entomol. Exp. Appl* 88: 255–263.
[45] M Boisvert, J Boisvert, **2000.** *Biocont. Sci. Technol* 10: 517–561.
[46] L S He, K H Ong, **2000**. *Singapore Journal of Primary Industries* 28: 7-12.
[47] R Bernotienė , **2001**. *Norwegian Journal of Entomology*. Vol. 48: 155–120p.
[48] D Damiens, G Martin, P Deswattines, T Hance, **2011**. *Faunistic Entomology* 63 (3):157-163.
[49] L J Goldberg, J Margalit, **1977**. *Mosquito News* 37: 355-358.
[50] H de BARJAC , **1978**. Toxicité de *Bacillus thuringiensis* var. *israelensis* pour les larves d'*Aedes aegypti* et d'*Anopheles stephensi*. C. R. Acad. Sci. (Paris) 286 D: 1175-1178.
[51] Ö Östman, J O Lundström, T Z P Vinnersten, **2008**. *Hydrobiologia* 607: 231–235.
[52] S S Gill, E A Cowles, P V Pietrantonio, **1992**. *Ann. Rev. Entomol* 37: 615-636.
[53] L S Bauer, **1995**. *Florida Entomol* 78(3): 414- 442.
[54] M P Meadows, **1993**. *Bacillus thuringiensis* in the environment: Ecology and risk assessment. In *Bacillus thuringiensis, an Environmental Biopesticide: Theory and Practice* (ed. P. F. Entwistle, J. S. Cory, M. J. Bailey and S. Higgs). John Wiley & Sons 193-220pp.
[55] R Garcia, B D Rochers, W Tozer, B Des Rochers, **1980.** Studies on the toxicity of *Bacillus thuringiensis* var. *israelensis* against organisms found in association with mosquito larvae. *Proceedings and papers of the Forty eighth Annual Conference of the California Mosquito and Vector Control Association, Inc.* (Grant, C. D., eds.), Anaheim, California 33-36.

[56] R W Merritt, M Wipfli, **1994.** Impact of *Bti* on non-target aquatic insects and trout. Michigan State University Http://www.msue.msu.edu/msue/imp/modet/61893002.html.
[57] T Caquet , M Roucaute, P Le Goff, L Lagadic, **2011**. *Ecotoxicology and Environmental Safety* 74: 1122–1130.
[58] N Becker, J Margalit, **1993**. Use of *Bacillus thuringiensis israelensis* against mosquitoes and blackflies. In: Entwistle, P.F., Cory, J.S., Bailey, J.M., Higgs, S. (Eds.), *Bacillus thuringiensis*, an Environmental Biopesticide: Theory and Practice. John Wiley and Sons, Ltd 149–170pp.
[59] A E Hershey, A R Lima, G J Niemi, R R Regal, R, **1998**. *Ecol. Appl* 8: 41–60
[60] P B Barnes, M G Chapman, **1998**. Effects of the larvicide (Vectobac) on assemblages of benthic invertebrates in Bicentennial Park. Centre for Research on Ecological Impacts of Coastal Cities, Sydney.
[61] C S Charbonneau, R D Drobney, C F Rabeni, **1994**. *Environ. Toxicol. Chem* 13: 267–279.
[62] L Lagadic, TH Caquet, D Fourcy, M Heydorff, **2002**. Evaluationa long terme des effets de la démoustication dans le Morbihan. Suivi de l'impact écotoxicologique des traitements sur les invertébrés aquatiques entre 1998 et 2001. Scientific Report. Research Agreement Conseil Général du Morbihan 215p.
[63] T Z P Vinnersten, J O Lundström, E Petersson, J Landin, **2009**. *Hydrobiologia* 635 : 189–203.
[64] J O Lundström, Y Brodin, M L Schäfer, T Z Persson Vinnersten, Ö Östman, **2010a**. *Bull. Entomol. Res* 100: 433–444.
[65] J O Lundström, M L Schäfer, E Petersson, T Z Persson Vinnersten, J Landin, Y Brodin, **2010b**. *Bull. Entomol. Res* 100: 117–125.
[66] M S Mulla, B A Federici, H A Darwazeh, **1982**. *Environ Entomol* 11:788–795.
[67] J Boisvert, J O Lacoursiere, **2004**. Le *Bacillus thuringiensis israelensis* et le contrôle des insectes piqueurs au Québec. Envirodoq No. ENV/2004/0278. Ministere de l'Environnement, Quebec.
[68] World Health Organization, **1999**. Microbial pest control agent : *Bacillus thuringiensis*. Finland, WHO publications 99 pp.
[69] G J Niemi, A E Hershey, L Shannon, J M Hanowski, A Lima, R P Axler, R R Regal, **1999**. *Environ. Toxicol. Chem* 18 : 549–559.
[70] E H Abdel-Rahman, M A Hassanian, **1999**. *Beni-Suef. Vet. Med. J* 9: 137-148.
[71] M A El-Emam; S El-Hak, M Shafik, **1996b**. *Egypt. J. appl. Sci* 11 (9): 184-191.
[72] G Y Osman, A M Mohamed, A Abdel Kader, A A Mohamed, **2011.** *Journal of Applied Pharmaceutical Science* (10): 47-55.
[73] K N Abdel-Megeed, M M Abd El-Aziz, **1999**. *Egypt. J. Zool* 33: 267-275.
[74] R L Norland, M S Mulla, **1975**. *Environ Entomol* 4: 145–152.
[75] M Car, F C de Moor , **1984**. *Onderstepoort J Vet Res* 51:155–160.
[76] C Back, J Boisvert, J O Lacoursiere, G Charpentier, **1985**. *Can Entomol* 117:1523–1534.
[77] I Ishaaya, **2001**. Biochemical sites of insecticide action and resistance. *Springer-Verlag Berlin Heidelberg* 293-321.
[78] Y Wei, A G Appel, W J Moar, N Liu N, **2001**. *Pest. Manag. Sci* 57 : 1055-1059.
[79] A A Enayati, H Ranson, J Hemingway, **2005**. *Insect. Molec. Boil* 14(1): 3-8.
[80] X Li , M A Shuler, M R Berenbaum, **2007**. *Ann. Rev. Entomol* 52 : 231-253.
[81] T H Chen, P Tang, C F Yang, L H Kao, Y P Lo, **2011**. *Virology* 410: 410-417.
[82] D G Clark, **1989**. *Comp. biochem. Physiol* 92B: 419-449.
[83] S Boyer, M Paris, S Jego, G Lempérière, P Ravanel, **2012**. *Biological Control* 62 (2): 75-81p.
[84] C C Lin, C F Yang, C H Tu, C G Huang, Y T Shih, C K Chuang, W J Chen, **2007**. *Virus Res* 124: 176-183.
[85] I M Dubovskiy, V V Martemyanov, Y L Vorontsova, M J Rantala, E V Gryzanova, V V Glupov, **2008**. Effect of bacterial infection on antioxidant activity and lipid peroxidation in the midgut of *Galleria mellonella* L. larvae (Lepidoptera, Pyralidae). *Comparative Biochemistry and Physiology Part C* 148: 1–5.
[86] C Pasay, L Arlian, M Morgan, R Gunning, L Rossiter, D Holt, S Walton, S Beckham, J McCarthy, **2009**. *PLoS Negl Trop Dis* 3**:**e354.
[87] K E Mounsey, C J Pasay, L G Arlian, M S Morgan, D C Holt, B J Currie, S F Walton and J S McCarthy, **2010**. *Parasites & Vectors*, 3:43.
[88] C R Lauzon, S E Potter, R J Prokopy, **2003.** *Environ. Entomol* 32: 953-962.
[89] O Duron, P Labbe, C Berticat, F Rousset, S Guillot, M Raymond, M Weill, **2006.** *Evolution* 60: 303-314.
[90] S Kontsedalov, E Zchori-Fein, E Chiel, Y Gottlieb, M Inbar, M Ghanim, **2008.** *Pest Manag. Sci* 64: 789-792.