Research Journal of Agriculture and Biological Sciences, 6(6): 823-828, 2010

Toxicity of Thiamethoxam, Behavioral Effects and Alterations in Chromatin of A*pis mellifera* L., 1758 (Hymenoptera; Apidae)

[1]José Ricardo Penteado Falco, [1]Joliano Hideo Hashimoto, [1]Fábio Fermino,
[2]Vagner Alencar Arnaut de Toledo

[1]Departamento de Biologia Celular e Genética (CCB) - Bloco H67. Universidade Estadual de Maringá. Avenida Colombo, 5790. CEP 87020-900. Maringá, Paraná, Brazil.
[2]Departamento de Zootecnia (CCA). Universidade Estadual de Maringá, Paraná, Brazil.

Abstract: The great use of insecticides is affecting as much the insect's curses as other organisms. The insecticide Thiamethoxam is a composed neonicotinoid of high toxicity on bees. These works aimed at characterize in the chromatin of Malpighian tubules of worker's *Apis mellifera* with different ages the effect of this insecticide. Besides, the behavior of the same ones was analyzed, that it based on the observation of acceptance/rejection of the food and of the rate of the worker's mortality. For the analysis of the chromatin the technique of Critical Electrolytes Concentration (CEC) were undertaken with Toluidine Blue staining (TB) at pH 4.0. It is sensitive to differentiate types of complex DNA-proteins in chromatin *in situ* and *in vitro*. Behavioral differences, alterations in the values of CEC and chromatin structure were observed at different ages as different concentrations of the insecticide used, evidencing the toxicity of the same, mainly in low concentrations (sublethal doses).

Key words: bioindicators, citochemistry, insecticide, behavior.

INTRODUCTION

Modern farming techniques have greatly increased production levels, but they have also expanded the areas of agricultural cultivation, agricultural were multiplied and the natural habitats of the pollinator were destroyed affecting the populations negatively[13].

Many methods, physical, chemical and biological, are used to determine the presence of residues of pesticides[29]. The bioassay need to present simplicity and adaptability to be accepted in the analysis of the residues[18]. The bees are susceptible to many insecticides commonly used to protect crops. These insects could be used as bioindicators for the determination of residues of some insecticides, besides detecting the toxicity level for the bee's front to insecticides commonly used[38,1,28].

A simple way, of low cost and efficient to determine the presence of residues of insecticides in the farming it is the use of the honeybees as bioindicators. The effect of several pesticides was already studied in bees[18,26,28], suggesting that those insects can indicate the presence of residues of chemical products.

Insects have Malpighian tubules in digestory system, they are organs excretory with independent functional status[31,14,17,527]. These tubules possess microvillus with great increment in the superficial area for passive and active transport of substances including salts minerals, water, products of the metabolism (composed nitrogenous, sulfur and phosphate), acids, bases and some ions[33], but even detoxicate the organism from pesticides[19].

Stain molecules link themselves to phosphate groups available in DNA and RNA in tissues stained with Toluidine Blue (TB) solutions at pH 3.6-4.0. Both are stained with TB solutions[16]. Non-protein DNA complex and protein-DNA complexes in the chromatin of somatic cells generally present violet-colored metachromatic basophilia. Metachromasy occurs when TB molecules, linked to phosphate groups of nucleic acids, are piled up and interact among themselves [36,6,22].

If TB staining occurs in the presence of Mg^{2+} ions, competitions ensues through DNA and RNA negative charges. In the case of specific Mg^{2+} ion concentration, staining of the nucleic acid will fail to be metachromatic. The phenomenon has been called critical electrolyte concentration (CEC) by Vidal and Mello[21]. It is expressed by salt mol and visually recognized by its chromatin greenish color. The spectral curve of TB-stained chromatin absorption peaks indicates that metachromasy (highest availability of free phosphate groups) has an absorption peak at λ

Corresponding Author: José Ricardo Penteado Falco, Departamento de Biologia Celular e Genética (CCB) - Bloco H67. Universidade Estadual de Maringá. Avenida Colombo, 5790. CEP 87020-900. Maringá, Paraná, Brazil.
E-mail: jrpfalco@uem.br

= 540-550 nm with violet staining. On the other hand, its erasure indicates λ = 625-630 nm with green staining. Actually, this fact characterizes CEC without the use of microspectrophotometry[22].

CEC research with TB staining and Mg^{2+} as an inorganic ion, which was originally used for *in vitro* protein-DNA models[35], was efficient to differentiate types of protein-DNA complexes *in situ* chromatin [22,20,34,8].

There is a variation in the competition between TB and Mg^{2+} for linking sites in chromatin when heterochromatin and euchromatin, spermatozoa of different animal species, DNA and RNA puffs, bands of polytenic chromosomes and the same nucleus under different physiological conditions or development, and metabolic stress are compared. Differences in the availability of phosphate groups in nucleic acid and the packaging of the above determine the degree of condensation and changes in CEC values. Condensed chromatin has a higher CEC rate than that in de-condensed chromatin[21,20,23,22,34,9,8]. Chromatin condenses and de-condenses in proportion to the rate DNA specific cell sequences are accessed for genes[2].

Were analyzed the effects of the insecticide Thiamethoxam on workers of *Apis mellifera*. We determined the values of the critical electrolyte concentration (CEC) in the chromatin of cells from Malpighian tubules of worker's at *Apis mellifera* with different ages. Also were verified if the bees had change of behavioral after Thiamethoxam exposure and if that affects the chromatin of Malpighian tubules and consequently the CEC values.

MATERIALS AND METHODS

Adult workers of bees *Apis mellifera* were used with different ages (0, 7, 14 and 21 days after emersion) maids in the apiary of FEI – Fazenda Experimental de Iguatemi-UEM, Maringá PR, Brazil. To know the age (0, 7, 14 and 21 days) of the bees, they were marked. The pictures selected with it creates to emerge were involved with paper and taken for a greenhouse at 35ºC with relative humidity of 80%. As soon as the bees emerged were marked in the thorax with colored paint no poisonous and returned for the beehive.

The bioassay were mounted in cardboard boxes with dimensions 12 x 12 x 12cm tends in one on their sides a screen for the aeration of the insects, and a feeder in interior. In each box 20 bees were maintained in greenhouse with temperature and humidity controlled. Bioassays were accomplished with different concentrations of the insecticide Thiamethoxam (Novartis®) dissolved in water. In the tests workers of bees were used with ages of 0, 7, 14 and 21 days after emersion. Each assay was developed in the following way: the adult worker's imprisonment for 24 hours in the boxes tests with feeder containing a honey mixture and solution with the insecticide in the proportion of 1:1, in the concentrations of $6x10^{-3}$; $3x10^{-3}$; $1,5x10^{-3}$; $5x10^{-4}$; $5x10^{-5}$; $5x10^{-6}$ mg/ml (dose recommended by the manufacturer is from $1x10^{-1}$ to $2x10^{-1}$mg/ml to tomato crops).

The behavior analysis was accomplished according to observation of the acceptance or rejection of the solution honey/insecticide and tax (%) of mortality of the bees in the different ages.

Malpighian tubules of *Apis mellifera* were extracted and cut in a physiological solution for insects (1.8 g NaCl; 1.88 g KCl; 0.16 g CaCl; 0.004 g $NaHCO_3$; distilled water - q.s.p. 100 ml); ground (lamina and slide) in acetic acid 45% and frozen in liquid nitrogen. The slide was removed when room temperature was reached. The material was then fixed in ethanol: acetic acid (3:1 v/v) for 2 min; washed for 10 min in ethanol 70%; and stained for 20 minutes with TB 0.025% in a MacIlvaine buffer pH 4.0, without $MgCl_2$. Other laminas were also stained with TB 0.025% in a MacIlvaine buffer, pH 4.0, with different $MgCl_2$ concentrations (0.02; 0.05; 0.08; 0.10; 0.12; 0.15; 0.20; 0.30 mol/L). Material was then washed for 5 seconds in de-ionized water and air dried. Xylol bleaching was undertaken for 15 minutes and mounted on Entellan sheet[36]. After drying, laminas were analyzed and photographed under a Zeiss standard light microscope (Carl Zeiss, Jena, Germany).

RESULTS AND DISCUSSION

Behavior analysis revealed that the bees consume a larger amount of mixture honey/insecticide in the smallest concentrations of the insecticide. There is a larger mortality of bees when these are fed with the solution containing low concentrations of the insecticide, being larger the mortality of younger bees (Table 1). Bees contract the abdomen some time after having ingested the mixture, as a colic, being disoriented, regurgitating the consumed mixture. They died presenting the extended proboscis and protracted legs.

When insecticide was in larger concentrations bees didn't feed. When the concentration of the insecticide is smaller bees consume the polluted honey and some die (Table 1).

Residues of this insecticide in the cultures can contaminate and kill a lot of bees, if the same ones don't detect it and if they feed of pollen and/or nectar contaminated, could cause serious damages in the pollination.

Food polluted, with low concentrations of the insecticide, can be collected and carried to the beehive and intoxicating a larger number of individuals.

Table 1. Mortality (%) of bees, *Apis mellifera*, after the honey ingestion containing insecticide Thiamethoxam® in the different concentrations.

Concentration (mg/ml)	Age (Day)			
	0	7	14	21
$5x10^{-6}$	0%	12,5%	1%	5%
$5x10^{-5}$	83,3%	16%	30%	15%
$5x10^{-4}$	50%	53,8%	80%	95%
$6x10^{-3}$	100%	60%	90%	72,7%
$3x10^{-3}$	100%	95%	80%	90%
$1.5x10^{-3}$	100%	90%	95%	70%

This could to take serious problems like decrease of the pollinator number, could affect the fecundation of plants, interfering in perpetuation and in the quality of their products.

If the food be contaminated with high insecticide concentrations this can act as repellent, impeding that the pollinator usually executes his task. Therefore, production of fruits and/or seeds can be prejudiced.

Results of bioassay with confined beehives and semi-confined in tents, made possible to verify that *A. mellifera* is constituted an efficient agent in the pollination of the guarana tree (*Paulinia cupana* var. *sorbilis*), making possible a medium production of 2,3kg of dry grains for plant, while in the plants of the treatment total (exclusion of insects) the medium production was of 0,25kg[12], demonstrating the economical importance of the pollination.

The action of the insecticides organophosphorate inhibits cholinesterase and consequent acetylcholine accumulation, taking the subsequent stoppage of the muscles, impeding the breathing and provoking the death for anoxia. There are typical symptoms of contamination: regurgitation, disorientation, languidness, permanence in the beehive waiting for the paralysis and death. The wings stay moved away of the body, usually interlaced. A high percentile of the poisoned bees dies inside from beehive; bees poisoned by insecticides present as characteristic extended proboscis and legs protracted[3,39,40].

According to Hashimoto and cols.[15] in electrophoreses analyses there was a decrease in the activity of esterases 1, 2, 4 and 5 due to the contact and oral toxicity of the insecticide Thiamethoxam, that suggest that alteration in the activity of those isozymes can be used to discover the presence of residues of insecticides. In Attencia and cols.[4] the Esterases 3 and 4 of *Apis mellifera* from 1 to 14 days after insecticide application was used as methyl-parathion and Malathion bioindicators.

For the citochemistry analyses (Tables 2, 3 and 4) the values of CEC, for euchromatin and for heterochromatin are larger in the oldest, and smaller in youngest bees. Alterations in the values of CEC reflect structural changes in chromatin. Chromatin more coiled possesses CEC value higher than chromatin uncoiled, due to the interaction that the molecules of Toluidine blue suffer when closes or piled up. The proximity or piling up of the molecules of Toluidine blue cause increase of the metachromasy and, consequently, larger needed of inorganic cations to abolish it, e.g., higher CEC value[22,23,10,9]. As bees get older can be observed that there is a quick condensation of the chromatin, which can be demonstrated by the increase of the CEC value (Table 2). This increase in the value of CEC is due that there is a larger need of Mg^{2+} so that the coloration of the nucleic acid stops being metachromatic (violet). When a smaller amount of Toluidine blue is linked to the substratum the chromatin will present a greenish coloration (CEC). This coiled chromatin in relation to the aging is probably related to the several activities exercised by the bees in the different ages. Some examples of the tasks accomplished by the bees in the different ages are: about the 9 days of age, cleaning of cells, with 15-20 days construction of honeycombs, first flight and food collection about the 25-60 days of age[38].

In Malpighian tubules treated with the insecticide a smaller basophilic was observed, maybe by smallest amount of RNA. We believed that the presence of the insecticide in gastrointestinal treatment of bees can stops accomplishing normal functions to accomplish other, metabolize the insecticide.

The coiled of the chromatin is a lot of times accompanied by the gen inactivity[2,11]. Being compared CEC value in treatments with insecticide in different concentrations and group controls (no treated with insecticide) it could be observed that in the bees with 7 days of age the heterochromatin suffered a reduction in CEC value (uncoiled of the chromatin) (Table 2 and 3). Euchromatin suffered increase of CEC value (coiled of the chromatin) only in the concentration 10^{-5} mg/ml (table 2 and 3); in bees with 14 days of age there was increase in values of heterochromatin and euchromatin (table 2, 3 and 4). In bees with 21 days of age there was reduction in heterochromatin (table 3 and 4).

Table 2: Answers of the Nuclear basophilic and critical electrolyte concentration (CEC) in the chromatin of Malpighian tubules from *Apis mellifera* after treatment with TB 0.025% in MacIlvaine buffer pH 4.0 added of $MgCl_2$ in various concentrations.

Treatment	Age (days)					
	7		14		21	
	He[1]	Eu[2]	He	Eu	He	Eu
TB	Vi[3]	Vi	Vi	Vi	Vi	Vi
TB + $MgCl_2$ 0.02 Mol/l	Vi	Vi	Vi	Vi	Vi	Vi
TB + $MgCl_2$ 0.05 Mol/l	Vi	Gr	Vi	Bl / Gr	Vi	Bl
TB + $MgCl_2$ 0.08 Mol/l	Vi	Vi	Vi	Gr	Bl	Gr
TB + $MgCl_2$ 0.10 Mol/l	Vi / Bl[4]	Bl / Gr	Vi / Bl	Bl / Gr	Bl	Bl
TB + $MgCl_2$ 0.12 Mol/l	Bl	Vi	Bl / Gr	Gr	Vi	Bl / Vi
TB + $MgCl_2$ 0.15 Mol/l	Gr[5]	Gr	Gr	Bl	Bl	Gr
TB + $MgCl_2$ 0.20 Mol/l	Vi	Vi	Gr	Gr	Gr	Gr
CEC value (Mol/l)	0.15	0.05	0.15	0.08	0.2	0.08

[1] He: heterochromatin. [2] Eu: euchromatin. [3] Vi: violet. [4] Bl: blue. [5] Gr: green.

Table 3: Nuclear basophilic answers and critical electrolyte concentration (CEC) in the chromatin of Malpighian tubules from *Apis mellifera* with 7, 14 and 21 days of age fed with solution honey/Thiamethoxam in different concentrations treated with TB 0.025% in MacIlvaine buffer pH 4.0 added of $MgCl_2$ in various concentrations.

Treatment	Age (days)																	
	7						14						21					
	He[1]	Eu[2]	He	Eu	He	Eu	He	Eu	He	Eu	He	Eu	He	Eu	He	Eu	He	Eu
TB	Vi[3]	Vi	Vi	Vi	Vi	Vi	Vi	Vi	Vi	Vi	Vi	Vi	Vi	Vi	Vi	Vi	Vi	Vi
TB + $MgCl_2$ 0.02Mol/l	Vi	Bl/ Gr	Vi	Vi/ Bl	Vi	Vi/ Bl	Vi	Bl	Vi	Vi/ Bl	Vi	Vi	Vi	Vi	Vi	Vi/Bl	Vi	Vi
TB + $MgCl_2$ 0.05 Mol/l	Vi/ Bl	Gr	Vi/ Bl	Bl	Vi/ Bl	Gr	Vi	Bl	Vi/ Bl	Bl / Gr	Vi	Bl	Vi	Vi/ Bl	Vi	Vi/Bl	Vi	Bl/ Gr
TB + $MgCl_2$ 0.08 Mol/l	Vi/ Bl	Bl/Gr	Vi/ Bl	Gr	Bl/ Gr	Bl / Gr	Vi	Bl	Vi/ Bl	Gr	Vi/ Bl	Vi/ Bl	Vi/ Bl	Bl	Vi	Vi	Vi/ Bl	Gr
TB + $MgCl_2$ 0.10 Mol/l	Bl[4]	Bl	Bl	Gr	Bl	Gr	Vi/ Bl	Gr	Bl	Bl	Bl	Gr	Vi	Bl	Vi	Gr	Vi/ Bl	Vi
TB + $MgCl_2$ 0.12 Mol/l	Gr[5]	Bl	Bl	Gr	Gr	Bl / Gr	Bl	Bl /Gr	Bl /Gr	Bl/Gr	Bl/Gr	Gr	Bl/ Gr	Gr	Vi/ Bl	Gr	Vi/ Bl	Bl
TB + $MgCl_2$ 0.15 Mol/l	Bl	Gr	Gr	Bl	Bl	Bl	Bl	Vi/ Bl	Gr	Gr	Gr	Gr	Bl	Bl	Vi	Bl	Gr	Gr
TB + $MgCl_2$ 0.20 Mol/l	Bl	Bl	Bl	Bl	Bl	Bl	Gr	Bl	Bl	Bl	Bl	Gr	Gr	Bl	Gr	Bl	Bl	Gr
CEC value Mol/l	0.12	0.1	0.15	0.1	0.12	0.05	0.2	0.1	0.15	0.08	0.15	0.1	0.2	0.12	0.2	0.1	0.15	0.1
	$5x10^{-6}$		$5x10^{-5}$		$5x10^{-4}$		$5x10^{-6}$		$5x10^{-5}$		$5x10^{-4}$		$5x10^{-6}$		$5x10^{-5}$		$5x10^{-4}$	
											insecticide concentration (mg/ml)							

[1] He: heterochromatin. [2] Eu: euchromatin. [3] Vi: violet. [4] Bl: blue. [5] Gr: green.

Table 4: Nuclear basophilic answers and critical electrolyte concentration (CEC) in the chromatin of Malpighian tubules from *Apis mellifera* with 14 and 21 days of age fed with solution honey/Thiamethoxam in different concentrations treated with TB 0.025% in MacIlvaine buffer pH 4.0 added of $MgCl_2$ in various concentrations.

Treatment	Age (days)											
	14						21					
	He[1]	Eu[2]	He	Eu	He	Eu	He	Eu	He	Eu	He	Eu
TB	Vi[3]	Vi	Vi	Vi	Vi	Vi	Vi	Vi	Vi	Vi	Vi	Vi
TB + $MgCl_2$ 0.02 Mol/l	Vi	Bl	Vi	Vi/Bl	Vi	Vi	Vi	Vi	Vi	Vi/Bl	Vi	Vi
TB + $MgCl_2$ 0.05 Mol/l	Vi	Bl	Vi/Bl	Bl/Gr	Vi	Gr	Vi	Vi/Bl	Vi	Vi/ Bl	Vi	Bl/Gr
TB + $MgCl_2$ 0.08 Mol/l	Vi	Bl	Vi/Bl	Gr	Vi/Bl	Vi/Bl	Vi/Bl	Bl	Vi	Vi	Vi/Bl	Gr
TB + $MgCl_2$ 0.10 Mol/l	Vi/ Bl	Gr	Bl	Bl	Bl	Gr	Vi	Gr	Vi	Gr	Vi/Bl	Vi
TB + $MgCl_2$ 0.12 Mol/l	Bl[4]	Gr	Bl /Gr	Bl/Gr	Bl/Gr	Gr	Bl/Gr	Gr	Vi/ Bl	Gr	Vi/Bl	Bl

Table 4: Continue

TB + $MgCl_2$ 0.15 Mol/l	Bl	Vi/ Bl	Gr	Gr	Gr	Gr	Bl	Bl	Vi	Bl	Gr	Gr
TB + $MgCl_2$ 0.20 Mol/l	Gr[5]	Bl	Bl	Bl	Bl	Gr	Gr	Bl	Gr	Bl	Bl	Gr
CEC value Mol/l	0.2	0.12	0.15	0.1	0.15	0.1	0.2	0.1	0.2	0.1	0.15	0.1
	6×10^{-3}		3×10^{-3}		15×10^{-4}		6×10^{-3}		3×10^{-3}		15×10^{-4}	
	Insecticide concentrations (mg/ml)[1]											

He: heterochromatin. [2]Eu: euchromatin. [3]Vi: violet. [4]Bl: blue. [5]Gr: green.

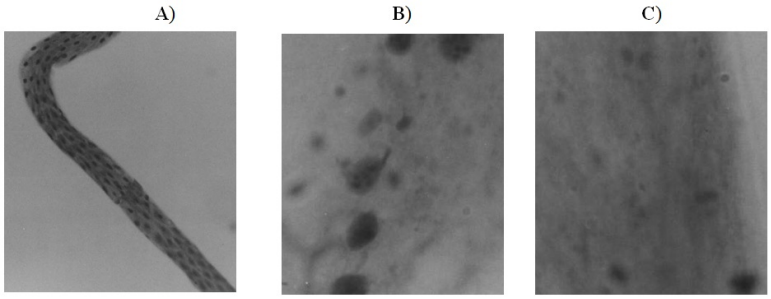

Fig. 1: Malpighian tubules of *Apis mellifera* (7 days of age) treated with TB added of $MgCl_2$. (A) TB treatment, 826x. (B) TB treatment, 4135x. (C) TB + $MgCl_2$ 0,15Mol/l, CEC value, 4135x.

The obtained results demonstrated that different ages and concentrations of the insecticide used produce relative condensation of the chromatin. Results indicate that Thiamethoxam is poisonous to *Apis mellifera*, affecting her nervous system and her normal behavior. This allows suggesting that characteristics as regurgitation of the consumed food, disorientation, extended proboscis and legs protracted. Individuals died can be used as indicators of the contamination of bees by Thiamethoxam.

The citochemistry analyses demonstrated that Thiamethoxam affects the structure of chromatin in Malpighian tubules of *A. mellifera* and their CEC value can be altered not only for the action of the insecticide, but also age or activity exercised by the bee.

REFERENCES

1. Accort, M., R. Guarcini & L.P. Oddo, 1992. The bee as a biological indicator and test insect. Redia, 74: 1-5.
2. Alberts, B., A. Johnson, J. Lewis, M. Raff, K. Roberts & P. Water. 2004. Biologia Molecular da Célula. 4ª ed. Artmed, Porto Alegre.
3. Atkins, E.L., 1997. Injury to honey bees by poisoning. In: GRAHAM, J.M. The hive and the honey bee. Michigan: Bookcrafters, pp: 1324.
4. Attencia, V.M., M.C.C. Ruvolo-Takasusuki, V.A.A. Toledo. 2005. Esterases Activity in *Apis mellifera* After Exposure to Organophosphate Insecticides (Hymenoptera: Apidae). Sociobiology, v. 45(3): 587-595.
5. Chapman, R.F., 1998. The insects: structure and functions, 4th ed. Cambridge University Press, Harvard.
6. Chayen, J. & L. Bitensky, 1991. Analysis of chemical components of cell and tissues. Reactions for nucleic acids and polyphosphate. In: Chayen, J. & L. Bitensky. Practical histochemistry. 2a Ed. New York. John Wiley & Sons., pp: 77-98.
7. Erickson Jr., E.H., B.J. Erickson & J.A. Wyman, 1994. Effects on Honey Bees of Insecticides Applied to Snap Beans in Wisconsin: Chemical and Biotic Factors. Journal of Economic Entomology, 87(3): 596-600.
8. Falco, J.R.P. & M.L.S. Mello, 1999. Critical electrolyte concentration of spermatozoal chromatin containing histone H1 variants. Genet. Mol. Biol., 22(2): 197-200.
9. Falco, J.R.P., 1999.Caracterização citoquímica de complexos DNA-proteína contendo variantes de histona H1. Tese de doutorado. UNICAMP/IB, Campinas, São Paulo.
10. Falco, J.R.P., M.L.S. Mello, S.S. Maria & N.A. Grazziotin, 1999. Critical electrolyte concentration of chicken erythrocyte chromatin. Acta Histochem. Cytochem., 32(1): 73-76.
11. Felsenfeld, G., 1992. Chromatin as an essential part of the transcriptional mechanism. Nature, 355: 219-224.
12. Ferreira, M.N., E.N. Marques, R.D. Miyazaki, 2000. Polinização dirigida com *Apis mellifera* L. sobre a produção de guaraná, *Paulinia cupana* var. *sorbili*s. In: Anais Congresso Brasileiro de Apicultura. Florianópolis, SC, pp: 13.

13. Freitas, B.M., 2000. Polinização de fruteiras tropicais. In: Annais Congresso Brasileiro de Apicultura. Florianópolis, SC, pp: 13.
14. Gillott, C., 1980. Entomology. Plenum Press, New York.
15. Hashimoto, J.H., M.C.C. Ruvolo-Takasusuki & V.A. Arnaut de Toledo, 2003. Evaluation of the Use of the Inhibition Esterases Activity on *Apis mellifera* as Bioindicators of Insecticide Thiamethoxam Pesticide Residues. Sociobiology, 42(3): 693-700.
16. Lison, L. Histochimie et cytochimie anymales. Paris, Gauthier-Villarrs. 1960.
17. Maddrell, S.H. & M.J. O'donnell, 1992. Insect Malpighian tubules: V. ATPase action in ion and fluid transport. J. Exp. Biol., 172: 417-429.
18. Mansour, S.A., 1987. Is it possible to use the honey bee adult as a bioindicator for the detection of pesticide residues in plants? Acta Biol Hung., 38(1): 69-76.
19. Mcgettigan, J., R.K.J. Mclennan, K.E. Broderick, L. Kean, A.K. Allan, P. Cabrero, M.R. Regulski, V.P. Pollock, G.W. Gould, A. Davies & J.A. Dow. 2005. Insect renal tubules constitute a cell-autonomous immune system that protects the organism against bacterial infection. Insect Biochem. Mol. Biol., 35: 741-754.
20. Mello, M.L.S. & J.R.P. Falco, 1996. Critical electrolyte concentration of DNA-protein complexes in spermatozoal and somatic cell nuclei of the honey bee, *Apis mellifera*. Insect Biochem. Mol. Biol., 26(8-9): 793-795.
21. Mello, M.L.S. & B.C. Vidal, 1989. Critical electrolyte concentration of the heterochromatin and euchromatin of *Triatoma infestans*. Cytobios, 59 (237): 87-93.
22. Mello, M.L.S., 1997. Cytochemistry of DNA, RNA and nuclear proteins. Braz. J. Genet., 20(2): 257-264.
23. Monteiro, A.L.P. & M.L.S. Mello, 1998. Critical electrolyte concentration of chromatin in polytene chromosomes of *Trichosia pubescens* (Diptera, Sciaridae). Genetics Mol. Biol., 21(2): 185-90.
24. Mota, M.O.S., R.H. Nogueira-Couto & D.T. Malerbo-Souza, 1996. Polinização e uso de atrativos para abelhas *Apis mellifera* em cultura de morango (*Fragaria x ananassa* D.). In: Annais Encontro Sobre Abelhas. Ribeirão Preto, Sâo Paulo.
25. Nogueira-Couto, R.H. & L.A. Couto, 1996. Apicultura: manejo e produtos. Jaboticabal: FUNEP. pp: 154.
26. Novartis Biociência S.A. In http://www.novartis.com.br. Access in August 2008.
27. O'Donnell, M.J. & J.H. Spring, 2000. Modes of control of insect Malpighian tubules: synergism, antagonism, cooperation and autonomous regulation. J. Insect Physiol., 46: 107-117.
28. Porrini, C., G. Celli & P. Radeghieri, 1998. Monitoring of pesticides through the use of honeybees as bioindicators of the Emilia-Romagna coastline (1995-1996). Annali di Chimica, 88: 243-252.
29. Rissato, S.R., M.S. Galhiane, F.R.N Knoll, R.M.B. de Andrade & M.V. de Almeida, 2006. Multiresidue method for monitoring environmental contamination by pesticides in the Bauru region (SP) using honey as bioindicator. Química Nova., 29(5): 950-955.
30. Scott, J.E., 1960. Aliphatic ammonium salts in the assay of acidic polysaccharides from tissues. In: Methods of Biochemical Analysis, vol 8, ed. by D. Glick. New York. Intersciense Publ., pp: 145-197.
31. Snodgrass, R.E., 1984. Anatomy of the honey bee. Cornell University Press, New York.
32. Sommer, P.G., 1997. Aspectos da apicultura brasileira. In: SIMPÓSIO PARANAENSE DE APICULTURA, 12. Anais... Guarapuava: Federação Paranaense de Apicultura, pp: 17-19.
33. Southwick, E.E., 1997. Physiology and social physiology of the honey bee. In: GRAHAM, J.M. The hive and the honey bee. Michigan: Bookcrafters.
34. Taboga, S.R., M.L.S. Mello, J.R.P. Falco, B.C. Vidal, 1996. Cytochemistry and polarization microscopy of amphibian sperm cell nuclei presumed to differ in basic protein composition. Acta Histochem. Cytochem., 29(2): 129-134.
35. Vidal, B.C. & M.L.S. Mello, 1989. Critical electrolyte concentration of DNA and nucleoprotein complexes *in vitro*. Acta Histochem. Cytochem., 22(4): 471-478.
36. Vidal, B.C., 1987. Métodos em Biologia Celular. In: Vidal, B.C. & Mello, M.L.S. Biologia Celular. Rio de Janeiro. Atheneu, pp: 5-39.
37. Ware, G.W., 1994. The pesticides book. 4th Ed. Thomson Publications, Fresno, California.
38. Winston, M.L., 1987. The age-related activities of worker bees. In: Winston, M.L. The biology of the honey bee. Cambridge: Harvard University Press.
39. Wise, H., 2000. Apicultura - novos tempos. Guaíba: Agropecuária.
40. Wolff, F.B., 2000. Efeitos dos agrotóxicos sobre a apicultura e a polinização de soja, citros e macieira. In: Annais Congresso Brasileiro de Apicultura. Florianópolis, Santa Catarina.