Vol.55, n. 1: pp. 47-53, January-February 2012
ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY
A N I N T E R N A T I O N A L J O U R N A L

Side-Effects of Pesticides Used in the Organic System of Production on *Apis mellifera* Linnaeus, 1758

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ABSTRACT

This study aimed to evaluate the effects of pesticides, used in the organic system, on Apis mellifera *under laboratory conditions. Four multiple (0.25x, 0.5x, 1x and 2x) concentrations as recommended by they manufacturers of the following products: Rotenat CE®, Pironat®, Biopirol 7M®, Organic neem®, Natuneem® and lime sulfur were tested by topical application and ingestion. Of all the products and concentrations tested, only the lime sulfur (5000 ml 100L^{-1} and 10000 mL 100L^{-1} of water) by ingestion, and Rotenat CE® (1200ml 100L^{-1} of water) on topical application were considered slightly harmful for* A. mellifera, *as the classification of IOBC/WPRS for the laboratory tests.*

Key words: Honeybees, rotenone, lime sulfur, neem, pyroligneous extract

INTRODUCTION

The emergence of new production systems, such as organic, which aim for the sustainability, while preserving or increasing the biological diversity, and minimization of all the forms of contamination, makes it critical to search for alternative techniques of control, selective and not harmful, especially on the populations of beneficial insects, such as natural enemies and pollinators. Among the possible alternatives for the pests and diseases controlling in the organic agriculture, there are products such as oils, plant extracts and syrups. However, for most of these substances, there is no scientific evidence regarding the efficiency on the pests and sustainability to the system, especially if they are selective to beneficial insects. Knowledge of the effects that pesticides have on beneficial insects, seeking the use of selective products is crucial for the sustainability of farming systems (Croft 1990).

Pollination has great importance in agricultural ecosystems (Free 1993; Roubik 1995). Bees are the most important flower visitors; they are responsible for pollinating more plant species than any other group (Raven et al. 2001). According to FAO (2004) estimates, approximately 70% of cultivated plant species worldwide are pollinated by some the species of bee. Ollerton et al. (2011) estimated 78-94% of flowering species relying on the biotic pollination. In flowering period, in the crops of commercial value, the presence of bees promotes the increase in the production of fruits and seeds (Morgado et al. 2002). In orchards, 75-80% of harvests are usually due to pollination by this group of insects (Guimarães 1989). Among the species of bees, *Apis mellifera* L. (Hymenoptera, Apidae) stands out for being a

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generalist, with excellent adaptation to the American continent, being easily found in almost all the agricultural areas, the principal responsible by the pollination of many fruit plants (Carvalho 2006; James and Pitts-Singer 2008).

In selectivity studies on the terrestrial animals, two groups are used, birds and bees (Devillers 2002). Aside from their ecological importance, the honey bees are interesting test organisms because of their rearing and manipulation facility, caused by relatively homogeneous individuals, by the general knowledge about their biology and, by presenting a life cycle, relatively short. These characteristics made *A. mellifera* the most widely used because of its extensive worldwide distribution (Devillers 2002). According to the author, all the insecticides used in agriculture should be tested on their bees to estimate the ecotoxicology, using different methodologies, depending on the purpose of the study.

For registration of new pesticides, the toxicological evaluation of *A. mellifera*, in several countries, including Brazil, is based on the protocols established by the European agencies (OECD 1998a, b; OEPP/EPPO 1992, 1993, 2001a, b) and the U.S. Environmental Protection Agency (U.S. EPA 1996a, b). Thus, this study aimed to evaluate, through the topical application and ingestion, the insecticidal effect of the pesticides used in organic production system on workers of *A. mellifera* under laboratory conditions.

MATERIALS AND METHODS

General experimental procedures

Bioassays to assess the action of the pesticides on *A. mellifera* were performed according to the methods of testing for exposure through oral and contact protocols established by the "Organisation for Economic Cooperation and Development (OECD 1998a, b) and the results were classified according to criteria of the IOBC/WPRS (International Organization for Biological Control) for laboratory tests on beneficial organisms (Hassan et al. 1985; Hassan et al. 1992) into: 1) harmless (mortality <30%), 2) slightly harmful (> 30% and <79%), 3) moderately harmful (> 80% and <99%), and 4) harmful (>99%). The experiment was conducted in an environmental room at 29 ± 1ºC, 70 ± 10% R.H., without photophase. For each product the concentrations described in Table 1 were used, and as a control, distilled water and fenthion (50 g $100L^{-1}$) were used. Bioassays were conducted under a completely randomized design with three replicates per treatment/concentration.

Table 1 - Products and concentrations evaluated on *Apis mellifera* in the laboratory.

Product	Commercial name	Product Information	Concentrations used (mL $100L^{-1}$ of water) *	Manufacturer
Neem Oil	Organic neem®	80% neem oil	125, 250, **500**, 1000	Dalquim
Neem Oil	Natuneem®	1500 ppm azadirachtin	125, 250, **500**, 1000	Natural Rural
Rotenone	Rotenat ®	extract of *Derris* spp. with 5% rotenone	150, 300, **600**, 1200	Natural Rural
Lime sulfur	---	20% S + 9% Ca	1250, 2500, **5000**, 10000	Sul Fertilizantes
Pyroligneous extract	Pironat®	N.I.	62,5, 125, **250**, 500	Natural Rural
Pyroligneous extract	Biopirol 7M®	N.I.	50, 100, **200**, 400	Biocarbo
Fenthion	Lebaycid 500 CE®	500 ppm a.i.	**100**	Bayer Cropscience

* The concentration recommended by the manufacturers in bold.
N.I. Not informed by the manufacturer

Colony of *A. mellifera*

The workers of *A. mellifera* used in the experiments were collected from the central comb of a rational beehive American type, with no evidence of contamination by the pathogens, maintained at the experimental site of the Department of Phytosanity of UFRGS. The hive was kept as recommended for honey production (Pereira et al. 2003). A protein paste comprising soy flour, granulated sugar and honey (3:1:1, respectively) and a sugar and water based syrup (1:1) were fed for the colony weekly (directly into coverage feeder).

Contact action in *Apis mellifera*

To assess the topical action by contact of the products, about 30 honeybees workers were removed and placed directly in the cages for the test made of PVC pipe (12 cm diameter x 8 cm in height), closed at one end with wire screen of 16 mesh /cm² and on the other, a foam (density 33 and 4 cm in height), according to a methodology proposed by Sattler et al. (1990). In the laboratory, the honeybees workers were anesthetized by cold at -12°C for up to three minutes and then subjected to topical application using 1μL of test solution on the dorsum of the thorax employing 1mL microsyringe coupled to a manual micro-applicator (Burkard Manufacturing Co. Ltd.). The products were applied in four concentrations (Table 1). The workers bees were fed with distilled water, soaked in cotton wool and a piece of crystallized honey (10 g), provided in a circular plastic container (3 x 2 cm). The cages were kept in an environmental room.

Ingestion action in *A. mellifera*

The bioassay to evaluate the action by ingestion of the products was carried out following the protocols of OECD (1998b). About 30 honeybee's workers per cage were deprived of food for two hours before the treatments. The products were offered at a solution of 50% sucrose, at the concentrations used for testing topical application (Table 1), through a strip of fabric Spontex Resist® inserted into a glass tube of 20 mL. After four hours, the treatment solution was removed and replaced, *ad libtum*, by an aqueous solution only of sucrose (50%).

Evaluation of bioassays on *A. mellifera*

The percentage of surviving individuals was recorded in each treatment after 24 and 48 h, considering those bees as dead which didn't show any movement when touched. The survival values were corrected by the formula of Abbott (1925) and the variation in the percentage of surviving insects were subjected to variance analyses of repeated measures, using SPSS® (Statistical Package for Social Science release 15.0 – SPSS Inc., Chicago, IL). The averages were compared for the significance by Tukey test, with 5% significance of error. Finally, the values were evaluated as parameters of toxicity of the IOBC (International Organisation for Biological Control) (Hassan et al. 1985; Bakker et al. 1992; Hassan 1994, Reis et al. 1998).

RESULTS AND DISCUSSION

The product Rotenat CE® (1200 ml $100L^{-1}$) was the only one to cause significant mortality by topical application, resulting in survival of 80.2 ± 7.70% of *A. mellifera* 24 h after the treatment. However, this product was significantly less toxic to the bees than fenthion control (Table 2). Within 48 h after application, this product also showed significant effects, with a survival of 69.5 ± 6.67% of the bees. This value despite being significantly higher than the control with fenthion, was very close to the threshold than the IOBC considered as a product slightly harmful (mortality between > 30% and < 79%) (Hassan et al. 1985; Bakker et al. 1992; Hassan 1994; Reis et al. 1998).

There was a toxic effect of lime sulfur at 5000 ml $100L^{-1}$ and 10000mL $100L^{-1}$ on the workers of *A. mellifera* after 48 h (Table 2). The effect at the highest dose despite being below to the IOBC regarded as slightly harmful, also was a value very close to the threshold, and therefore, the solution would be to follow the sequential schedule of evaluation, conducting the tests in semi-field conditions.

The other products tested by the topical application did not affect the honeybees survival at any dose and any evaluation period (Table 2).

The absence of deleterious effect of neem on *A. mellifera* at high concentrations had been reported by Schmutterer (1990), with commercial Margosan-O®, through direct contact. A similar result was obtained by González-Gómez et al. (2006) evaluating the toxicity of neem seed extract and a commercial product (Neem PHC®) on *A. mellifera*. However, this product could have an effect on the immature stages, as azadirachtin, the main component of the extract acted by interfering in the functioning of the endocrine glands that control metamorphosis in the insects (Viegas Jr. 2003).

A significant increase in the mortality of *A. mellifera* at different concentrations of the Natuneem®, Organic neem®, lime sulfur and Pironat® (Table 2) were recorded in the present study between 24 and 48 h. This indicated that if they were followed for a longer period, these products could cause toxicity on *A. mellifera*. However, because of the difficulty of keeping the bees alive for long periods outside the hive, the assessments after 48 h were not done.

Table 2 - Average percentage of workers survival (± S.D.) of *Apis mellifera*, 24 and 48 hours after treatment by topical contact (29 ± 1 ºC, 70 ± 10% RH, without photophase).

Treatments		Conc.[1]	R.D. [2]	Time (hours)			
				24		48	
Product	Commercial name						
Neem Oil	Organic neem®	125	0.25	98.6±1.73	Aa[3]	95.2±3.62	Aab
		250	0.5	98.3±2.11	Aa	94.8±4.02	Aab
		500	1	98.6±1.67	Aa	93.6±3.83	Bab
		1000	2	98.7±1.62	Aa	98.5±1.61	Aab
Neem Oil	Natuneem®	125	0.25	98.8±2.06	Aa	93.2±0.46	Bab
		250	0.5	100±0.00	Aa	93.1±1.98	Bab
		500	1	98.8±1.92	Aa	89.0±1.00	Bab
		1000	2	97.3±2.31	Aa	87.7±0.70	Bab
Rotenone	Rotenat®	150	0.25	98.7±2.15	Aa	87.6±5.67	Bab
		300	0.5	96.0±3.87	Aa	87.2±5.47	Aab
		600	1	94.1±5.16	Aa	86.8±4.75	Aab
		1200	2	80.2±7.70	Ab	69.5±6.67	Ad
Lime sulfur	---	1250	0.25	98.9±1.75	Aa	92.5±5.57	Aab
		2500	0.5	98.0±1.91	Aa	91.7±3.69	Bab
		5000	1	92.7±5.17	Aa	83.4±3.38	Bbc
		10000	2	91.6±4.01	Aa	74.1±2.82	Bdc
Pyroligneous extract	Pironat®	62.5	0.25	100±0.00	Aa	95.2±2.02	Bab
		125	0.5	100±0.00	Aa	92.6±2.13	Bab
		250	1	98.8±1.99	Aa	90.7±2.10	Bab
		500	2	98.7±2.30	Aa	87.9±3.60	Bab
Pyroligneous extract	Biopirol 7M®	50	0.25	96.9±3.03	Aa	94.9±4.63	Aab
		100	0.5	96.7±3.33	Aa	94.4±5.09	Aab
		200	1	94.5±5.20	Aa	92.5±7.04	Aab
		400	2	94.9±4.45	Aa	89.9±5.98	Aab
Fenthion	Lebaycid 500 CE®	100	-	0±0.00	Ac	0±0.00	Ae
Control	-	-	-	99.2±1.66	Aa	98.9±1.92	Aa

[1] Conc. = Concentration (mL of commercial product per 100L of water).
[2] R.D. = multiples of the recommended concentration by the manufacturer.
[3] Averages followed by distinct uppercase letters in line and lowercase in column differing among them by Tukey's test at 5% significance of error.

Results on the action by ingestion of the products at 24 h showed that only lime sulfur (10000mL $100L^{-1}$) differed from the control with water (Table 3). However, after 48 h, several products and doses differed from the control, especially the lime sulfur (5000mL $100L^{-1}$ – 69.9±2.99%; and 10000 mL $100L^{-1}$ – 31.7±1.51%) and Rotenat CE® (1200 mL $100L^{-1}$ – 76.6±2.89%), which presented higher toxicity level, with the exception of control with fenthion. This was similar to what occurred in the topical test, but with smaller percentages of survival. This could be due to a differentiated action of the exposure method, with a higher toxicity when the product was ingested than by the topical contact. Hoskins and Gordon (1956) considered that the cuticle penetration and the effectiveness of the insecticides decreases. Croft (1990) reported that in general the contamination by ingestion was higher.

The Biopirol 7M® and products based on neem caused significant mortality at the doses recommended by the manufacturers, which could be due to some antifeeding effect, mainly from neem whose main component (azadirachtin) showed a phagodeterrent effect on the insects (Godfrey 1994; Martinez 2002; Viegas Jr. 2003; Isman 2006).

Table 3 - Average percentage of workers survival (± S.D.) of *Apis mellifera*, 24 and 48 hours after treatment by ingestion (29 ± 1 ºC, 70 ± 10% RH, without photophase).

Treatments		Conc.[1]	R.D.[2]	Time (hours)			
Product	Commercial name			24		48	
Neem Oil	Organic neem®	125	0.25	98.8±2.06	Aa[3]	94.3±2.25	Aab
		250	0.5	100±0.00	Aa	93.0±2.00	Bab
		500	1	98.8±1.99	Aa	88.9±0.85	Bbc
		1000	2	97.3±2.32	Aab	83.9±0.64	Bc
Neem Oil	Natuneem®	125	0.25	98.8±1.99	Aa	93.9±1.84	Bab
		250	0.5	97.5±2.26	Aab	89.4±0.77	Bbc
		500	1	96.5±3.45	Aab	88.8±2.39	Bbc
		1000	2	95.4±1.79	Aab	83.7±2.39	Bc
Rotenone	Rotenat®	150	0.25	98.7±2.16	Aa	96.4±3.68	Aa
		300	0.5	98.5±2.24	Aa	94.9±2.28	Aab
		600	1	95.5±4.13	Aab	89.2±3.71	Abc
		1200	2	93.3±0.65	Aab	76.6±2.89	Bd
Lime sulfur	---	1250	0.25	98.8±2.06	Aa	94.4±1.70	Bab
		2500	0.5	98.0±1.39	Aa	88.8±1.89	Bbc
		5000	1	93.7±2.08	Aab	69.9±2.99	Be
		10000	2	90.9±5.47	Ab	31.7±1.51	Bf
Pyroligneous extract	Pironat®	62.5	0.25	100±0.00	Aa	97.6±2.10	Ba
		125	0.5	100±0.00	Aa	96.0±0.34	Ba
		250	1	100±0.00	Aa	94.3±1.93	Bab
		500	2	98.7±2.22	Aa	93.0±0.24	Bab
Pyroligneous extract	Biopirol 7M®	50	0.25	98.8±2.06	Aa	94.3±2.25	Aab
		100	0.5	100±0.00	Aa	93.0±2.00	Bab
		200	1	98.8±1.99	Aa	88.9±0.85	Bbc
		400	2	97.3±2.32	Aab	83.9±0.64	Bc
Fenthion	Lebaycid 500 CE®	100	-	0±0.00	Ac	0±0.00	Ag
Control	-	-	-	100±0.00	Aa	99.3±0.80	Aa

[1] Conc. = Concentration (mL of commercial product per 100L of water).
[2] R.D.= multiples of the recommended concentration by the manufacturer.
[3] Averages followed by distinct uppercase letters in line and lowercase in column differing among them by Tukey's test at 5% significance of error.

There was a significant decrease in the survival between 24 and 48 h for most products and doses, suggesting that the toxic effects for ingestion was over a longer period of time. The control reference insecticide (fenthion - 50g $100L^{-1}$) caused the death of 100% of the honeybees at first evaluation 24 h after the application for both the methods of exposure tested., Aguiar-Menezes (2005) found rotenone as non-toxic for the bees. However, the results of this study and classification of IOBC demonstrated the need for more tests on *A. mellifera*, using other methods of exposure and especially in conditions of semi-field.

Hunt et al. (2003) found the products based on rotenone, azadirachtin and sulfur, such as lime sulfur relatively non-toxic to *A. mellifera*. However, to Silva (2005) and Dal Soglio et al. (2007) found lime sulfur causing a great impact on this insect's fauna in the area of fruit plants such as citrus. The authors found a quantitative reduction in the taxa and a decrease in the abundance of those less frequently caught in McPhail trap in citrus orchards and considered that this reduction could be related to the number of applications of the lime sulfur, since at higher intensities higher impacts were observed.

The results obtained in this study raised issues concerning the marketing and correct use of pesticides which have been used routinely in the organic production system, because it could be harmful to the pollinators such as *A. mellifera*. The results indicated that it was appropriate to avoid the application of the products such as lime sulfur and rotenone at the times of flowering in the culture, native vegetation and surrounding areas with the presence of bees, as they might be toxic to

them. Also, further studies would be needed on the effects of the products on the immature stages of *A. mellifera*, and field tests by observing the behavior of the bee's orientation in the hive, in collecting nectar and in the presence of residues in the honey.

CONCLUSIONS

The following products were slightly harmful to *A. mellifera*, as the classification of IOBC/WPRS under laboratory tests: lime sulfur (5000mL $100L^{-1}$ and 10000 mL $100L^{-1}$ of water) by ingestion, and Rotenat CE® (1200 mL $100L^{-1}$ of water) on topical application.

ACKNOWLEDGEMENTS

Authors thank to the National Council for Scientific and Technological Development (CNPq) for scholarships granted to authors.

REFERENCES

Abbott WS. A method of computing the effectiveness of an insecticide. *J Econ Entomol.* 1925; 18: 265-267.

Aguiar-Menezes EL. Inseticidas botânicos: seus princípios ativos, modo de ação e uso agrícola. Seropédica: Embrapa Agrobiologia; 2005.

Bakker FM, Grove A, Blümel S, Calis J, Oomen P. Side-effect test for phytoseiids and their rearing methods. *IOBC/WPRS Bull.* 1992; 15: 61-81.

Carvalho SM. Toxicidade de produtos fitossanitários utilizados em citricultura à operárias de *Apis mellifera* Linnaeus, 1758 (Hymenoptera: Apidae). [Master Dissertation in Agronomy]. Lavras, Brasil: Universidade Federal de Lavras; 2006.

Croft BA. Arthropod biological control agents and pesticides. New York: Wiley; 1990.

Dal Soglio FK, Silva FF da, Jesus CR de, Redaelli LR, Koller OC, Greve C, et al. Desenvolvimento de tecnologias apropriadas para a produção de mudas e frutas cítricas em sistema familiar de produção. *Rev. Bras. Agroecol.* 2007; 2(1): 98-101.

Devillers J. The ecological importance of honey bees and their relevance to ecotoxicology. In: Devillers J, Pham-Delègue MH. Honey Bees: estimating the environmental impact of chemicals. London: Taylor & Francis, 2002. p. 1-10.

FAO. Conservation and management of pollinators for sustainable agriculture – the international response. In: Freitas BM, Pereira JOP. Solitary bees: conservation, rearing and management for pollination. Fortaleza: Imprensa Universitária; 2004. p. 19-22.

Free JB. Insect Pollination of Crops. London: Academic Press, 1993.

Godfrey CRA. Agrochemical from Natural Products. New York: Marcel Dekker Inc., 1994.

González-Gómez R, Otero-Colina G, Villanueva-Jiménez JA, Pérez-Amaro JA, Soto-Hernández RM. Toxicidad y repelencia de *Azadirachta indica* contra *Varroa destructor* (Acari: Varroidae). *Agrociencia*. 2006; 40: 741-751.

Guimarães NP. Apicultura a ciência da longa vida. Belo Horizonte: Itatiaia, 1989.

Hassan SA. Guidelines for testing the effects of pesticides on beneficial organisms: description of test methods. *IOBC/WPRS Bull.* 1992; 15(3): 18-39.

Hassan SA. Comparison of three different laboratory methods and one semi-field test method to assess the side effects of pesticides on *Trichogramma cacoeciae*. *IOBC/WPRS Bull.* 1994; 17: 133-141.

Hassan SA, Bigler F, Blaisinger P, Bogenschütz H, Brun J, Chiverton P, et al. Standard methods to test the sideeffects of pesticides on natural enemies of insects and mites developed by the IOBC/WPRS working group "Pesticides and Beneficial Organisms". *Bull EPPO*, 1985; 15: 214-255.

Hoskins WM, Gordon HT. Arthropod resistance to chemicals. *Annu Rev Entomol.* 1956; 1: 89-122.

Hunt G, Edwards R, Foster RE. Protecting honey bees from pesticides. Purdue University Cooperative Extension Service: Beekeeping: E-53-W, 2003.

Isman MB. Botanical insecticides, deterrents, and repellents in modern agriculture and increasingly regulated world. *Annu Rev Entomol.* 2006; 51: 45-66.

James R, Pitts-Singer TL. Bee pollination in agricultural ecosystems. New York: Oxford University Press, 2008.

Martinez SS. O nim - *Azadirachta indica*: natureza, usos múltiplos, produção. Londrina: IAPAR, 2002.

Morgado LN, Carvalho CF, Souza B, Santana MP. Fauna de abelhas (Hymenoptera: Apoidea) nas flores de girassol *Helianthus annuus* L., em Lavras - MG. *Cienc Agrotec.* 2002; 26(6): 1167-1177.

OECD. Guideline 214: Honeybees, acute contact toxicity test. OECD Guidelines for the testing of chemicals. 1998a [Accessed on Jun 2009] Available from: *www.oecd.org.*

OECD. Guideline 213: Honeybees, acute oral toxicity test. OECD Guidelines for the testing of chemicals. 1998b [Accessed on Jun 2009] Available from: *www.oecd.org.*

OEPP/EPPO. Guideline for the efficacy evaluation of plant protection products – Sideffects on honeybees – directive PP 1/170 (2). *Bull.OEPP/EPP*. 1992; 22: 203-216.

OEPP/EPPO. Decision making scheme for the environmental risk assessment of plant protection products. *Bull. OEPP/EPPO*. 1993; 23: 151-165.

OEPP/EPPO. Revised draft of EPPO Guidelines PP 1/170: Guidelines for the efficacy evaluation of plant protection products: sideeffects on honeybees. Appendix I, In: Belzunce, LP, Pélissier C, Lewi GB. Hazards of pesticides to bees. *Les Colloques de l'INRA*. 2001a; 98: 279-286.

OEPP/EPPO, EPPO. Decision Making Scheme: Decision-making scheme for the environmental risk assessment of plant protection products. Appendix II, In: Belzunces LP, Pélissier C, Lewis GB. Hazards of pesticides to bees. *Les Colloques de l'INRA*, 2001b; 98: 287-295.

Ollerton J, Winfree R, Tarrant S. How many flowering plants are pollinated by animals? *Oikos.* 2011; 120: 321-326.

Pereira FM, Lopes MTR, Camargo RCR, Vilela SLO. Produção de mel. Embrapa Meio-Norte. 2003. [Accessed in Jun 2009] Available from: *http://www.cpamn.embrapa.br/pesquisa/apicultura/mel/index.htm*

Raven PH, Evert RF, Eichhorn SE. Biologia Vegetal. Rio de Janeiro: Guanabara Koogan, 2001.

Reis PR, Chiavegato LG, Moraes GJ, Alves EB, Souza EO. Seletividade de agroquímicos ao ácaro predador *Iphiseiodes zuluagai* Denmark & Muma (Acari: Phytoseiidae). *An Soc Entomol Bras.* 1998; 27(2): 265-274.

Roubik DW. Pollination of cultivated plants in the tropics. *FAO Agric Serv Bull.* 1995; 118.

Sattler A, Muxfeld GOB, Tapia CE. Manutenção e estímulo de abelhas *Apis mellifera* sob diferentes tipos de alimentação. In: 8th Congresso Brasileiro de Apicultura, Resumos. Campos do Jordão, SP. 1990. p. 21.

Schmutterer H. Properties and potential of natural pesticides from the neem tree. *Annu Rev Entomol.* 1990; 35: 271-297.

Silva FF da. Espécies de moscas frugívoras (Diptera: Tephritidae e Lonchaeidae), quantificação de danos e avaliação de medidas para o seu manejo em pomares orgânicos de citros. [PhD Thesis in Fitossanidade] Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil. 2005.

US EPA. Ecological effects test guidelines OPPTS 850.3020 Honey bee acute contact toxicity test. *EPA.* 1996a; 712-C: 96-147.

US EPA. Ecological effects test guidelines OPPTS 850.3040 Field testing for pollinators, *EPA*. 1996b; 712-C: 96-150.

Viegas-Júnior C. Terpenos com atividade inseticida: uma alternativa para o controle químico de insetos. *Quim Nova*. 2003; 26(3): 390-400.

Received: October 05, 2010;
Revised: May 17, 2011;
Accepted: November 10, 2011.