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BIOASSAY OF INSECTICIDES AGAINST THREE HONEY BEE SPECIES IN LABORATORY CONDITIONS

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ABSTRACT. A study was conducted at the Eco-toxicology laboratory in the Department of Agricultural Entomology, University of Agriculture Faisalabad, against three species *Apis florea*, *A. dorsata and A. mellifera* of honey bees, to check long-term survival of honeybees when exposed to different insecticides. In this study, we used a modeling approach regarding survival data of caged bees under chronic exposure to seven insecticides (Carbosulfan, Chlorpyrifos, Bifenthrin, Spinosad, Indoxacarb, Emamectin benzoate and Imidacloprid), having three replicates and four concentrations (1000, 500, 250, 125 and 0 ppm). We demonstrate the chronic toxicity induced by these insecticides. Laboratory bioassay of these insecticides showed that carbosulfan and imidacloprid were the most toxic at their high dose (1000 ppm) with LT_{50} of 4 hours in each case for *A. mellifera*, chlorpyrifos and imidacloprid were the most toxic at their high dose (1000 ppm) with LT_{50} of 5 hours in each case for *A. florea* whereas chlorpyrifos was the most toxic at high dose (1000 ppm) with LT_{50} of 5 hours for *A. dorsata*. However, LT_{50} of spinosad was increased up to 18 hrs with decreasing concentrations at 125 ppm against *A. mellifera*, LT_{50} of spinosad was increased up to 15 hrs with decreasing concentrations at 125 ppm against *A. florea* as well as LT_{50} of spinosad and Emamectin benzoate was increased up to 20 hrs with decreasing concentrations at 125 ppm against *A. dorsata*. However, LT_{50} of all controlled species was 91-103 hrs.

Key words: Honey bees; Chlorpyrifos; Imidacloprid; Concentrations; Toxicity.

INTRODUCTION

Honey bees are essential to modern agriculture. Honey bees not only provide pollination services but also provide honey which is of great medicinal importance. These provide main pollination services to many field crops, vegetables and fruit crops. Without adequate population of honey

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bees the considerable production of these crops would be impossible. The role and economic value of honey bees as agricultural pollinators was estimated and caused out with conclusion that an increase of 1.5 to 5.7 social gains, annually was due to the use of full activities of these insects (Southwick and Southwick, 1992).

Several neonicotinoids, however, show very strong toxicity to pollinating insects and in particular to the honey bee (*A. mellifera* L.), causing also other effects which are seldom easily identifiable, such as behavioural disturbances, orientation difficulties and impairment of social activities (Bortolotti *et al.*, 2003; Decourtye *et al.*, 2004a; Decourtye *et al.*, 2004b; Desneux *et al.*, 2007; El Hassani *et al.*, 2008; Maini *et al.*, 2010). Although potential problems could be reduced by treating seeds and not spraying flowering crops (Tomlin, 2003), alarming bee mortalities, clearly due to the use of neonicotinoids either for seed dressing or crop spraying, were recorded in many countries during the past few years, and various limitation in their use were enforced (Greatti *et al.*, 2003; Colin *et al.*, 2004; Greatti *et al.*, 2006; Forster, 2009; Janke and Rosenkranz, 2009; Maini *et al.*, 2010; Marzaro *et al.*, 2011). An average of 41% out crossing in chili peppers whereas introduction of bees during flowering increased the seed and fruit size (Cruz *et al.*, 2005).

The toxicity of insecticides and fungicides to honey bees were claimed by manufacturers to be harmless or only slightly toxic to the bees was investigated. It is also pointed out that such pesticides can be dangerous to bees and other pollinators, if allowed to come in to contact with flowering crops (Abramson *et al.*, 1999; Chauzat *et al.*, 2006).

The extent of loss of honey bees by indiscriminate use of insecticides was studied and pointed out that insecticides were responsible for decrease in pollinators (Abramson *et al.*, 1999; Chauzat *et al.*, 2006). It, therefore, could be stated that services of honey bee in the field of pollination are worth full despite of their value as honey produced. The role played by honey bees in increasing the crop yield is 10-20 times greater than their values in honey production. Unfortunately this useful aspect of honey bee is being ignored in Pakistan. So for, our farmers are not fully aware of useful role of honey bees in pollination.

The comparative toxicity of six contact and six systemic organophosphorus insecticides against *A. dorsata* F., *A. florea* F. and *A. indica* F. was reported and the bee mortality on account of all the insecticides declined with the passage of time but insects by which its numbers are being kept in check (Wiggle insecticide remained highly toxic to the three species of the bees even after 48 hours of the treatments) (Decourtye *et al.*, 2005).

The contact toxicities for acetone formulations azinphosmethyl, carbaryl, methyl parathion and permethrin were established. These insecticides were applied to workers of Africanized and European honey bee. Africanized bees showed greater tolerance to all the chemicals except cabaryl. The order of toxicity of the compounds on the Africanized bees was permethrin > carbaryl > azinphosmethyl > methyl parathion and on the European honey bees, the order of toxicity was permethrin > azinphosmethyl > carbaryl > methyl parathion. The laboratory experiment was conducted to assess the danger of Acetamiprid, Clothianidin, Thiacloprid, and Thiamethoxam for the honey bee. Imidacloprid was not investigated because the scientific literature already has a large store of information (Bortolotti *et al.*, 2003; Maini *et al.*, 2010).

The injudicious uses of insecticides to control the pests have resulted in scarcity of pollinating agents especially the honey bees. Moreover, the insecticidal residues on bee flora is additional cause of bee's mortality (Haq and Gardezi, 1983).

The aim of present studies is not only the determination of the safest insecticides to the honey bees but also the evaluation of the dose of most commonly used insecticides which can control the insect pests and less harming to honey bees.

MATERIAL AND METHODS

These research studies were conducted at the Eco-toxicology laboratory in the Department of Agricultural Entomology, University of Agriculture, Faisalabad Pakistan during 2009. Honey bees *A. florea* and *A. dorsata* were collected from the field while *A. mellifera* was collected from apiary of Department of Agricultural Entomology, University of Agriculture, Faisalabad Pakistan and were kept in the cages measuring 2×4 feet and bees were fed on 50% sucrose solution. Seven insecticides viz., Advantage 20EC, Tracer 240SC, Bifenthrin 20EC, Chlorpyrifos 40EC, Confidor 200SL, Steward 150SC and Timer 1.9EC were used in this experiment along with concentrations (1000, 500, 250, 125 and 0 ppm) prepared in the acetone (*Table 1*).

Residual toxicity was studied with jars. In this method 5ml of insecticide concentration was added in the each jar with a dropper. After drying the jar in the air 10 honey bees' worker were introduced, along with one control jar of acetone. This was replicated three times. The mortality was determined after 3, 6, 12, 24 hours and so on. Honey bees were kept in cage for very shorter periods of time (upto 24 hours).

Methodology for assay was developed in the lab under the supervision of Dr. Muhammad Altaf Sabri, so there is no reference in this regard. The jars were placed at temperature 35±1^{0}C and 50±5% humidity. Dose mortality relationship was determined by Probit analysis (Finney, 1971).

Table 1 - List of insecticides with common names, trade names and recommended dose acre^{-1}

Trade name	Common name	Dose ml acre^{-1}
Advantage® 20 EC	Carbosulfan	500
Lorsban® 40 EC	Chlorpyrifos	800-1000
Talstar® 20 EC	Bifenthrin	250
Tracer® 240 SC	Spinosad	80
Steward® 150 SC	Indoxacarb	175
Timer® 1.9 EC	Emamectin benzoate	200
Confidor® 200SL	Imidacloprid	125

RESULTS

LT_{50} of *A. mellifera*

Table 2 shows the LT_{50} values for *A. mellifera* by exposing to the glass jars, treated with different insecticides concentrations.

LT_{50} was 4 hrs at 1000 ppm concentration of imidacloprid with fiducial limit, *p* value and standard error was 5.6-8.2, 0.08 and 0.6, respectively. LT_{50} was 4 hrs at 1000 ppm concentration of carbosulfan with fiducial limit, *p* value and standard error was 5.8-8.7, 0.11 and 0.69, respectively. LT_{50} was 5 hrs at 1000 ppm concentration of bifenthrin with fiducial limit, *p* value and standard error was 6.5-9.3, 0.38 and 0.72, respectively. LT_{50} was 6 hrs at 1000 ppm concentration of chlorpyrifos with fiducial limit, *p* value and standard error was 5.3-8.1, 0.41 and 0.71, respectively. LT_{50} was 7 hrs at 1000 ppm concentration of emamectin benzoate with fiducial limit, *p* value and standard error was 6.8-9.5, 0.90 and 0.60, respectively. LT_{50} was 9 hrs at 1000 ppm concentration of spinosad with fiducial limit, *p* value and standard error was 8.3-11.3, 0.06 and 0.77, respectively. LT_{50} was 10 hrs at 1000 ppm concentration of indoxacarb with fiducial limit, *p* value and standard error was 7.6-10.5, 0.87 and 0.72, respectively. LT_{50} was 96 hrs of control treatment. And at the concentration of 125 ppm LT_{50s} increased to 11 hrs, 12 hrs, 12 hrs, 18 hrs, 16 hrs, 13 hrs and 10 hrs of carbosulfan, chlorpyrifos, bifenthrin, spinosad, indoxacarb, emamectin benzoate and imidacloprid with standard error 1.3, 1.5, 1.6, 1.4, 1.3, 1.3 and 1.1, respectively.

LT_{50} of *A. florea*

Table 3 shows the LT_{50} values for *A. florea* by exposing to the glass jars, treated with different concentration of insecticides.

LT_{50} were 5 hrs at 1000 ppm concentration of chlorpyrifos with fiducial limit, *p* value and standard error was 5.3-8.1, 0.40 and 0.71, respectively, and imidacloprid with fiducial limit, *p* value and standard error was 5.6-8.12, 0.88 and 0.66, respectively. LT_{50} was 6 hrs at 1000 ppm concentration of bifenthrin with fiducial limit, *p* value and standard error was 6.5-9.4, 0.38 and 0.73, respectively. LT_{50} were 7 hrs at 1000 ppm concentration of carbosulfan with fiducial limit, *p* value and standard error was 6.4-7.7, 0.00 and 0.71, respectively, spinosad with fiducial limit, *p* value and standard error was 8.3-11.3, 0.21 and 0.80, respectively, and emamectin benzoate with fiducial limit, *p* value and standard error was 6.8-9.5, 0.90 and 0.70, respectively. LT_{50} was 8 hrs at 1000 ppm concentration of indoxacarb with fiducial limit, *p* value and standard error was 7.7-10.5, 0.87 and 0.72, respectively. LT_{50} was 96 hrs of control treatment. And at the concentration of 125 ppm LT_{50s} increased to 13 hrs, 12 hrs, 13 hrs, 15 hrs, 12 hrs, 14 hrs and 10 hrs of carbosulfan, chlorpyrifos, bifenthrin, spinosad, indoxacarb, emamectin benzoate and imidacloprid with standard error 0, 1.54, 1.62, 1.40, 1.31, 1.37 and 1.15, respectively.

LT_{50} of *A. dorsata*

Table 4 shows the LT_{50} values for *A. dorsata* by exposing to the glass jars, treated with different concentration of insecticides.

LT_{50} was 5 hrs at 1000 ppm concentration of chlorpyrifos with fiducial limit, *p* value and standard error was 5.7- 8.3, 0.12 and 0.65, respectively. LT_{50} were 7 hrs at 1000 ppm concentration of bifenthrin with fiducial limit, *p* value and standard error was 7.1- 9.9, 0.26 and 0.73, respectively, emamectin benzoate with fiducial limit, *p* value and standard error was 6.5-9.2, 0.25 and 0.66, respectively, and imidacloprid with fiducial limit, *p* value and standard error was 6.7-9.5, 0.52 and 0.72, respectively. LT_{50} was 8 hrs at 1000 ppm concentration of carbosulfan with fiducial limit, *p* value and standard error was 8.7-11.3, 0.40 and 0.64, respectively. LT_{50} was 9 hrs at 1000 ppm concentration of indoxacarb with fiducial limit, *p* value and standard error was 7.9 -11.1, 0.58 and 0.80, respectively. LT_{50} was 11 hrs at 1000 ppm concentration of spinosad with fiducial limit, *p* value and standard error was 9.6-12.9, 0.25 and 0.83, respectively. LT_{50} was 96 hrs of control treatment. And at the concentration of 125 ppm LT_{50s} increased to 14 hrs, 13 hrs, 19 hrs, 20 hrs, 17 hrs, 20 hrs and 16 hrs of carbosulfan, chlorpyrifos, bifenthrin, spinosad, indoxacarb, emamectin benzoate and imidacloprid with standard error 1.41, 1.33, 1.75, 1.45, 1.24, 1.21 and 1.22, respectively.

DISCUSSION

Insecticides are used on different crops to control insect-pests of different crops. Along with harmful insects these insecticides also kill the beneficial insects like honey bees.

The insecticides in our experiment were Advantage 10EC, Tracer 240SC, Bifenthrin 20EC, Chlorpyrifos 40EC, Confidor 200SL, Steward 150SC and Timer 1.9EC. The insecticide which has less LT_{50} was considered as the more toxic to the honey bee. Carbosulfan has less LT_{50} against *A. floria* and *A. mellifera* but against *A. dorsata* its toxicity increased with long exposure of time. There were no comparable data was found.

Table 2 - LT_{50} of *A. Mellifera*

Concen. / Treat.	LT_{50} (hours), Fiducial limit (95%), *p* value, Standard error															
	1000 ppm				500 ppm				250 ppm				125 ppm			
	LT_{50}	F.L	*P*	S.E	LT_{50}	F.L	*P*	S.E	LT_{50}	F.L	*P*	S.E	LT_{50}	F.L	*P*	S.E
Carbosulfan	4	5.8-8.7	0.11	0.69	7	7.5-10.5	0.49	0.76	9	10.8-15.7	0.01	1.2	11	12.1-17.4	0.04	1.3
Chlorpyrifos	6	5.3-8.1	0.41	0.71	7	6.6-9.7	0.31	0.76	10	10.5-15.6	0.00	1.3	12	11.5-17.4	0.38	1.5
Bifenthrin	5	6.5-9.3	0.38	0.72	7	8.5-12.4	0.19	1.00	10	11.5-16.8	0.02	1.3	12	12.9-19.3	0.02	1.6
Spinosad	9	8.3-11.3	0.06	0.77	12	10.4-14.7	0.07	1.09	14	13.5-19.8	0.01	1.6	18	12.6-18.2	0.01	1.4
Indoxacarb	10	7.6-10.5	0.87	0.72	12	8.7-12.3	0.27	0.91	13	12.2-17.7	0.00	1.4	16	14.6-19.7	0.17	1.3
Emamectin benzoate	7	6.8-9.5	0.90	0.60	9	8.5-12.1	0.11	0.91	11	11.2-15.9	0.01	1.1	13	12.3-17.4	0.01	1.3
Imidacloprid	4	5.6-8.2	0.08	0.6	7	6.5-9.3	0.12	0.70	8	7.9-11.7	0.00	0.9	10	8.8-13.3	0.00	1.1
Control	96	*	*	*	99	*	*	*	91	*	*	*	103	*	*	*

Table 3 - LT_{50} of *A. florea*

Concen. / Treat.	LT_{50} (hours), Fiducial limit (95%), *p* value, Standard error															
	1000 ppm				500 ppm				250 ppm				125 ppm			
	LT_{50}	F.L	*P*	S.E	LT_{50}	F.L	*P*	S.E	LT_{50}	F.L	*P*	S.E	LT_{50}	F.L	*P*	S.E
Carbosulfan	7	6.4-7.7	00	0.71	9	*	*	*	11	*	*	*	13	*	*	*
Chlorpyrifos	5	5.3-8.1	0.40	0.71	8	6.6-9.7	0.31	0.73	11	10.5-15.6	0.00	1.3	12	11.5-17.4	0.38	1.54
Bifenthrin	6	6.5-9.4	0.38	0.73	11	8.5-12.5	0.18	1.01	12	11.5-16.8	0.01	1.4	13	12.9-19.3	0.01	1.62
Spinosad	7	8.3-11.3	0.21	0.80	11	10.4-14.7	0.67	1.09	13	13.5-19.7	0.01	1.6	15	12.6-18.2	0.01	1.40
Indoxacarb	8	7.7-10.5	0.87	0.72	10	8.7-12.3	0.27	0.91	11	12.2-17.7	0.00	1.43	12	14.6-19.7	0.17	1.31
Emamectin benzoate	7	6.8-9.5	0.90	0.70	9	8.5-12.1	0.11	0.91	12	11.3-15.9	0.01	1.2	14	12.3-17.4	0.02	1.37
Imidacloprid	5	5.6-8.12	0.88	0.66	7	6.5-9.3	0.12	0.70	9	7.9-11.7	0.00	0.97	10	8.8-13.3	0.00	1.15
Control	96	*	*	*	99	*	*	*	91	*	*	*	103	*	*	*

Table 4 - LT_{50} of *A. dorsata*

Concen. / Treat.	LT_{50} (hours), Fiducial limit (95%), *p* value, Standard error															
	1000 ppm				500 ppm				250 ppm				125 ppm			
	LT_{50}	F.L	*P*	S.E	LT_{50}	F.L	*P*	S.E	LT_{50}	F.L	*P*	S.E	LT_{50}	F.L	*P*	S.E
Carbosulfan	8	8.7-11.3	0.40	0.64	10	9.3-12.2	0.31	0.76	12	13.4-18.8	0.01	1.37	14	14.8-20.3	0.01	1.41
Chlorpyrifos	5	5.7-8.3	0.12	0.65	7	7.5-10.5	0.48	0.77	11	11.5-5 16.6	0.01	1.29	13	13.1-18.3	0.02	1.33
Bifenthrin	7	7.1-9.9	0.26	0.73	12	10.1-13.8	0.41	0.98	14	14.6-20.8	0.02	1.59	19	16.6-23.4	0.06	1.75
Spinosad	11	9.6-12.9	0.25	0.83	13	11.9-15.9	0.24	1.00	15	15.3-20.8	0.06	1.42	20	16.4-22.1	0.12	1.45
Indoxacarb	9	7.9-11.1	0.58	0.80	11	9.6-12.9	0.25	0.83	13	12.2-16.3	0.14	1.07	17	14.2-19.1	0.21	1.24
Emamectin benzoate	7	6.5-9.2	0.25	0.66	11	9.9-13.7	0.06	0.94	13	12.3-16.7	0.07	1.11	20	15.1-19.8	0.41	1.21
Imidacloprid	7	6.7-9.5	0.52	0.72	10	8.3-11.8	0.22	0.89	12	11.3-15.5	0.03	1.09	16	13.1-17.9	0.10	1.22
Control	96	*	*	*	99	*	*	*	91	*	*	*	103	*	*	*

Bifenthrin is toxic to the all three species of honey bees with a low LT_{50}. Dai *et al.* (2010) used different pyrethroids including bifenthrin and their results showed that all these compounds were highly toxic to honey bees. These results were same as that work. Spinosad was also toxic to the honey bees and the LT_{50} is decreased with increased exposure of time which caused high mortality. Same results were found by (Miles, 2003), which showed that spinosad was highly toxic to the honey bees.

Emamectin benzoate and indoxacarb showed almost similar results that LT_{50} was high in start of experiments which decreased gradually with the passage of time, and cause increased mortality against all three species of honey bees. No comparable data was found.

Chlorpyrifos was highly toxic to the three species of honey bees which caused 100% mortality after 6 hrs at high concentrations (Johnson et al., 2010). They showed that chlorpyrifos was highly toxic to *A. cerana* species of honey bees.

Imidacloprid was recorded as highly toxic insecticide against *A. floria*, *A. mellifera* and *A. dorsata* which cause 100% mortality after 6hrs at high concentrations. No comparable results being noted.

The present results differ from those of (Abramson *et al.*, 1999; Chauzat *et al.*, 2006; Pistorius *et al.*, 2009).This difference may be due to the use of different insecticides on different species.

CONCLUSIONS

From these studies, it was concluded that all insecticides proved to be lethal for three species of honey bee. The results showed that *A. dorsata* had high LT_{50} than other two species *(A. florea* and *A. mellifera*). Confidor was proved to be more toxic with LT_{50} after 24 hrs 13, 13, and18 ppm for *A. florea, A. mellifera* and *A. dorsata*, respectively, followed by chlorpyrifos, while spinosad, indoxacarb and emamectin benzoate insecticides proved to be comparatively safer than other insecticides.

REFERENCES

Abramson C., Aquino I., Ramalho F., Price J., 1999 - The effect of insecticides on learning in the Africanized honey bee (*Apis mellifera* L.). Archives of Environmental Contamination and Toxicology 37, 529-535.

Bortolotti L., Montanari R., Marcelino J., Medrzycki P., Maini S., Porrini C., 2003 - Effects of sub-lethal imidacloprid doses on the homing rate and foraging activity of honey bees. Bulletin of Insectology 56, 63-68.

Chauzat M.-P., Faucon J.-P., Martel A.-C., Lachaize J., Cougoule N., Aubert M., 2006 - A survey of pesticide residues in pollen loads collected by honey bees in France. Journal of Economic Entomology 99, 253-262.

Colin M.E., Bonmatin J., Moineau I., Gaimon C., Brun S., Vermandere J., 2004 - A method to quantify and analyze the foraging activity of honey bees: relevance to the sublethal effects induced by systemic insecticides. Archives of

Environmental Contamination and Toxicology 47, 387-395.

Cruz D.d.O., Freitas B.M., Silva L.A.d., Silva E.M.S.d., Bomfim I.G.A., 2005 - Pollination efficiency of the stingless bee *Melipona subnitida* on greenhouse sweet pepper. Pesquisa Agropecuária Brasileira 40, 1197-1201.

Dai P.-L., Wang Q., Sun J.-H., Liu F., Wang X., Wu Y.-Y., Zhou T., 2010 - Effects of sublethal concentrations of bifenthrin and deltamethrin on fecundity, growth and development of the honeybee *Apis mellifera ligustica.* Environmental Toxicology and Chemistry 29, 644-649.

Decourtye A., Armengaud C., Renou M., Devillers J., Cluzeau S., Gauthier M., Pham-Delègue M.-H., 2004a - Imidacloprid impairs memory and brain metabolism in the honeybee (*Apis mellifera* L.). Pesticide Biochemistry and Physiology 78, 83-92.

Decourtye A., Devillers J., Cluzeau S., Charreton M., Pham-Delègue M.-H., 2004b - Effects of imidacloprid and deltamethrin on associative learning in honeybees under semi-field and laboratory conditions. Ecotoxicology and environmental safety 57, 410-419.

Decourtye A., Devillers J., Genecque E., Le Menach K., Budzinski H., Cluzeau S., Pham-Delègue M., 2005 - Comparative sublethal toxicity of nine pesticides on olfactory learning performances of the honeybee *Apis mellifera*. Archives of Environmental Contamination and Toxicology 48, 242-250.

Desneux N., Decourtye A., Delpuech J.M., 2007 - The sublethal effects of pesticides on beneficial arthropods. Annu. Rev. Entomol. 52, 81-106.

El Hassani A.K., Dacher M., Gary V., Lambin M., Gauthier M., Armengaud C., 2008 - Effects of sublethal doses of acetamiprid and thiamethoxam on the behavior of the honeybee (*Apis mellifera*). Archives of Environmental Contamination and Toxicology 54, 653-661.

Finney D., 1971 - Probit Analysis. Cambridge University Press. Cambridge, UK. p. 256.

Forster R., 2009 - Bee poisoning caused by insecticidal seed treatment of maize in Germany in 2008. Julius-Kühn-Archiv 423, 126-131.

Gallai N., Salles J.-M., Settele J., Vaissière B.E., 2009 - Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecological Economics 68, 810-821.

Greatti M., Sabatini A.G., Barbattini R., Rossi S., Stravisi A., 2003 - Risk of environmental contamination by the active ingredient imidacloprid used for corn seed dressing. Preliminary results. Bulletin of Insectology 56, 69-72.

Greatti M., Barbattini R., Stravisi A., Sabatini A.G., Rossi S., 2006 - Presence of the a.i. imidacloprid on vegetation near corn fields sown with Gaucho® dressed seeds. Bulletin of Insectology 59, 99-103.

Haq M., Gardezi T.H., 1983 - A comparative study on the toxicity of organophosphorus insecticides to honey bees. Journal of Pakistan Entomology 5, 83-87.

Janke M., Rosenkranz P., 2009 - Periodical honey bee colony losses in Germany: preliminary results from a four years monitoring project. Julius-Kühn-Archiv 423, 108-117.

Johnson R.M., Ellis M.D., Mullin C.A., Frazier M., 2010 - Pesticides and honey bee toxicity–USA. Apidologie Vol.41,No.3, May-June, 312-331.

Maini S., Medrzycki P., Porrini C., 2010 - The puzzle of honey bee losses: a brief review. Bulletin of Insectology 63, 153-160.

Marzaro M., Vivan L., Targa A., Mazzon L., Mori N., Greatti M., Petrucco Toffolo E., Di Bernard A., Giorio C., Marton D., 2011 - Lethal aerial powdering of honey bees with neonicotinoids from fragments of maize seed coat. Bulletin of Insectology 64, 119-126.

Miles M., 2003 - The effects of spinosad, a naturally derived insect control agent to the honeybee. Bulletin of Insectology 56, 119-124.

Oldroyd B.P., 2007 - What's killing American honey bees? PLoS biology 5(6): e168.

Pistorius J., Bischoff G., Heimbach U., Stähler M., 2009 - Bee poisoning incidents in Germany in spring 2008 caused by abrasion of active substance from treated seeds during sowing of maize. Julius-Kühn-Archiv 423, 118-126.

Southwick E.E., Southwick Jr. L., 1992 - Estimating the economic value of honey bees (Hymenoptera: Apidae) as agricultural pollinators in the United States. Journal of Economic Entomology 85, 621-633.

Tomlin C., 2003 -The Pesticide Manual. Alton, Hampshire, UK: Br. Crop Protection Council, 1344 p.p.