Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.5234

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **5 (4):** 641-646 (2017)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Sublethal Lethal Exposure of Certain Newer Insecticides Molecules to Honeybee, *Apis mellifera* Linnaeus

Ratnakar V.[1*], Koteswara Rao S. R.[2], Sridevi D. and Vidyasagar B.[1]

[1]Department of Entomology, [1]Department of Plant Pathology, College of Agriculture, Prof. Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad- 500 030, India

[2]Department of Entomology, S. V. Agricultural College, Acharya N.G. Ranga Agricultural University, Tirupati-517 502, India

*Corresponding Author E-mail: ratnakar.ento@gmail.com

Received: 17.07.2017 | Revised: 26.07.2017 | Accepted: 27.07.2017

ABSTRACT

Contact toxicity of nine newer insecticides viz., acetamiprid, clothianidin, imidacloprid, fipronil, thiamethoxam, spiromesifen, chlorantraniliprole, chlrofenapyr and diafenthiuron at half of the recommended dosewas tested on honeybee Apis mellifera L using the dry film method. Foragers were released into treated jars at different intervals i.e., 0, 12 and 24 hours after dry film formation. Found that spiromesifen and chlorantraniliprole relatively safer while clothianidin, actamiprid, thiamethoxam, imidacloprid and fipronil proved to be toxic and other insecticides i.e., diafenthiuron, chlorfenapyr were less toxic.

Key words: *Honeybee, Apis mellifera, Sublethal, Insecticides, Toxicity*

INTRODUCTION

The importance of bees lies not only in the production of honey, wax, pollen, propolis, royal jelly and venom, but also in the role they have in the pollination of entomophilous crops. The honey bee is credited with approximately 85% of the pollinating activity that ultimately enhances the production and productivity of the crop. It was found that growth in agricultural production achieved by the pollinating bees is 10 -15 times higher than the production of honey, wax, etc. Widespread use of pesticides in modern agriculture throughout the world has become necessary for the protection of crops against insect pests and diseases to avoid crop losses and to meet the food requirement of increasing population, but the injudicious use of pesticides has resulted in not only contamination of agroecosystem and agricultural produce, but also of nectar and pollen and caused heavy losses to honeybees and other pollinators. Unfortunately, these two practices are not always compatible, as honeybees are susceptible to many of commonly used pesticides[4,12]. Conservation of honeybees for crop pollination is vital to agricultural production[8]. In India, 90% of the pollination of crops grown across 50 million hectares was done by bees.

Cite this article: Ratnakar, V., Koteswara Rao, K.S.R., Sridevi, D. and Vidyasagar, B., Sublethal Lethal Exposure of Certain Newer Insecticides Molecules to Honeybee, *Apis mellifera* Linnaeus, *Int. J. Pure App. Biosci.* **5(4):** 641-646 (2017). doi: http://dx.doi.org/10.18782/2320-7051.5234

Although poorly studied, a harmonious compromise between pest management and honeybee pollination of crops in India is clearly important. Widespread use of pesticides in modern agriculture throughout the world has become necessary for protection of crops against insect pests and diseases to avoid crop losses and to meet the food requirement of increasing population, but the injudicious use of pesticides has resulted in contamination of not only the agroecosystem and agricultural produce, but also of nectar and pollen causing heavy losses to honeybees and other pollinators. Knowledge of relative safety of insecticides during flowering is essential to obtain maximum benefits from bee pollination. With this prelude, an experiment was conducted to evaluate the safety of certain insecticides to European honeybee, *Apis mellifera* L.

MATERIAL AND METHODS

Newer insecticides *viz.*, acetamiprid, clothianidin, imidacloprid, fipronil, thiamethoxam, spiromesifen, chlorantraniliprole, chlrofenapyr and diafenthiuronat half of the recommended dosewas tested on honeybee *Apis mellifera* L in the laboratory of Department of Entomology, College of Agriculture, Rajendranagar, Hyderabad during August 2014 to May 2015. Forager honeybees were collected at the entrance of the hive with the cone type muslin hand net (30 cm diameter), put in plastic jars and were immobilized by keeping them in deep freezers / refrigerator for about 5 minutes[10]. They were allowed to recover from cold treatment before exposure to insecticides. The test insecticides were evaluated for their toxicity by dry film method. In this method, half of the recommended doses of insecticides formulation were diluted in one liter of distilled water to make the spray solutions. One ml each of the respective test insecticide was transferred to a clean dry rearing jar of size 10 X 7 cm diameter. The jar was gently rotated and left for drying so that a thin dry film of the chemical was formed inside the jar. Ten forage adult bees were released into the each jar at different intervals *i.e.* 0, 12 and 24 hours of after dry film formation and within each interval, data on mortality of the bees was recorded at 2, 4, 6, 12, 24 and 48 hours. These intervals were considered since bees could be exposed to insecticides in the field any time after their spray and it is necessary to understand the residual toxicity of these insecticides on the bees. A cotton pad soaked in sugar solution (20%) was provided inside the jar and it was covered using muslin cloth. Each treatment was replicated thrice and a jar with a dry film of water served as control. The data obtained was analyzed statistically[11] using WINDOWSTAT package. Abbott's[2] formula was used to arrive at the corrected natural mortality. Bees were recorded as dead when gentle pressure upon the abdomen did not bring any twitching movement or positive response over a half an hour inspection period.

RESULTS AND DISCUSSION

Effect of certain newer insecticides at half of recommended dose on mortality of honeybees exposed 0 hours after dry film formation (Table: 1)

Among the treatments, significantly higher mortality of 79.99 per cent was recorded in clothianidin and thiamethoxam treatments. Diafenthiuron and spiromesifen recorded 10.00 and 4.76 per cent mortality, respectively and they were on par with each other. Chlorfenapyr did not record any mortality after 2 hours. But after 4 hours imidacloprid, acetamiprid and fipronil recorded 73.47, 66.73 and 63.39 per cent mortality, respectively.

Spiromesifen and chlorfenapyr recorded least per cent mortality of 10.02 each and they were on par with diafenthiuron (13.03%).After 6 hours, cent per cent mortality was recorded by clothianidin. Imidacloprid and acetamiprid caused 83.64 and 79.99 per cent mortality. Per cent mortality of 16.37 and 13.03 were recorded in diafenthiuron and spiromesifen, respectively which were on par with each other.

Among the treatments, after 12 hours cent per cent mortality were recorded by clothianidin and thiamethoxam and they were on par with imidacloprid (94.70%). Cent per cent mortality was registered by clothianidin, thiamethoxam, fipronil and acetamiprid after 48 hours. The order of per cent mortality of other treatments was imidacloprid > chlorantraniliprole > chlorfenapyr > diafenthiuron > spiromesifen. Chlorfenapyr, diafenthiuron and spiromesifen recorded 78.46, 55.62 and 44.41 per cent mortality, respectively after 48 hours.

Clothianidin recorded cent per cent mortality after 6 hours while thiamethoxam registered cent per cent mortality after 12 hours while acetamiprid and fipronil caused cent per cent mortality after 24 hours. Imidacloprid showed cent per cent toxicity after 48 hours. Spiromesifen was found to be least toxic with 44.41 per cent mortality.

Effect of certain newer insecticides at half of the recommended dose on mortality of honeybees exposed after 12 hours of dry film formation

Among the treatments, highest mortality was recorded by thiamethoxam(66.73%). Per cent mortality of 10.02 was observed in clothianidin, fipronil, imidacloprid, diafenthiuron and spiromesifen, while no mortality was recorded in acetamiprid, chlorantraniliprole and chlorfenapyr. After 4 hours, highest mortality was observed in thiamethoxam (79.99%). Clothianidin and imidacloprid recorded 60 and 30.01 per cent mortality, respectively. Chlorantraniliprole, spiromesifen, and fipronil recorded each 10.02 per cent mortality but were on par with chlorfenapyr (13.03%).After 6 hours, cent per cent mortality was recorded thiamethoxam. Acetamiprid and fipronil recorded lesser mortality of 26.53 per cent and 23.19 per cent, respectively which were on par with each other.

After 12 hours, the order of per cent mortality was thiamethoxam(100%) > clothianidin(78.47%) > acetamiprid(55.53%) = fipronil(55.53%) > imidacloprid (51.83%) > chlorfenapyr(44.32%) > diafenthiuron(22.20%) > spiromesifen (14.46%) > chlorantraniliprole(11.10%). Even after 24 hours of treatment the similar trend in mortality was observed in all the treatments. After 48 hours, cent per cent mortality was recorded bythiamethoxam and clothianidin. Lesser moratlity was observed in diafenthiuron (29.46%) and spiromesifen (22.20%) which were on par with each other while chlorantraniliprole caused least mortality (11.10%).

Thiamethoxam recorded highest mortality (100%) after 6 hours, while clothianidin registered cent per cent mortality after 48 hours indicating their toxicity to *A.mellifera.* Fipronil, imidacloprid and acetamipridwere moderately toxic while chlorfenapyr, spiromesifen, chlorantraniliprole and diafenthiuron were found to be less toxic to *A.mellifera.*

Effect of certain newer insecticides at half of the recommended dose on mortality of honeybees exposed after 24 hours of dry film formation

After 2 hours, highest mortality of 66.73 per cent was observed in thiamethoxam while 10.02 per cent mortality in each treatments of

diafenthiuron, imidacloprid and fipronil. Clothianidin, spiromesifen, acetamiprid, chlorantraniliprole and chlorfenapyr did not record any mortality. After 4 hours.lower per cent mortality of 10.02 was recorded in acetamiprid, diafenthiuron, fipronil and chlorfenapyr. After 6 hours acetamiprid, diafenthiuron and fipronil recorded each 20.01 per cent mortality. Chlorantraniliprole and spiromesifen recorded least mortality of honeybees (10.02%).

After 12 hours, highest mortality of 77.74 per cent was observed in each of thiamethoxam and clothianidin treatments. Spiromesifen and chlorantraniliprole exhibited 14.46 and 11.10 per cent mortality, respectively and were on par with each other.After 24 hours fipronil, imidacloprid, chlorfenapyr, acetamiprid, diafenthiuron, spiromesifen and chlorantraniliprole recorded 77.74, 70.48, 62.99, 36.94, 29.46, 22.21 and 14.46 per cent mortality, respectively. After 48 hours, diafenthiuron and spiromesifen showed 29.46 and 22.21 per cent mortality, respectively and they were on par with each other. Chlorantraniliprole recorded lowest mortality of 14.46 per cent.

Thiamethoxam and clothianidin registered cent per cent mortality after 24 hours, while none of the insecticides tested, recorded cent mortality even after 48 hours though imidacloprid was on par with them. Fipronil and chlorfenapyr seemed to be moderately toxic while chlorantraniliprole, diafenthiuron and spiromesifen were least toxic.

Results obtained in present study are in accordance with the findings of Pastagia and Patel[9] who reported that thiamethoxam caused 78.32 per cent mortality of honeybees at 0.005 per cent concentration.

Imidacloprid recorded cent per cent mortality after 48 hours. These results are in agreement with findings of Gradish *et al.* (2010) who found 80 per cent to 99 per cent mortality at 0.001 g l^{-1} and 0.1 g l^{-1} concentrations. Jeyalakshmi *et al.*[6] also found that at half of the recommended dose, clothianidin and thiamethoxam were more toxic than imidacloprid.

Chlorantraniliprole was found to be safe to bees at sublethal concentrations which is in agreement with Gradish *et al.*[5] who found that it was safe to at 0.001 g l^{-1} and 0.1 g l^{-1} concentrations.

Diafenthiuron recorded 10.00, 29.46 and 55.62 per cent mortality after 2, 24 and 48 hours, respectively. These results are in conformity with the findings of Aravind and Samiayyan *et al.*[3] who found 27.58 and 39.7 per cent mortality after 24 and 48 hours of treatment, respectively at 400 g a.i ha^{-1} .

Spiromesifen recorded 4.76, 13.03 and 22.21 per cent mortality after 2, 6 and 24, respectively. These results are in accordance with the findings of Kavitha *et al.*[7] who recorded 10.5 per cent mortality of bees after 6 hours of treatment at 96 g a.i. ha^{-1}. Vinothkumar *et al.*[13] observed 10 per cent mortality with spirotetramat (also derivative of tetronic acid) at 45g a.i ha^{-1} after 24 hours and his conclusion that spirotetramat is relatively safer to honeybees are also in support of the present studies.

The present study helped to understand the extent of safety of different insecticides to honey bees. Though insecticides are indispensable for crop protection, timing the sprays especially in the flowering stage of the crop helps avoid intervention with the natural activity of forager bees.

Table 1: Effect of certain newer insecticides at half of the recommended dose on mortality of European honeybee, *Apis mellifera* L.

Treatments	Dose (ml or g / l)	Per cent mortality of honey bees exposed Immediately,12 and 24 hours after dry film formation																	
		Immediately after dry film formation						12 hours after dry film formation						24 hours after dry film formation					
		2 hr	4 hr	6 hr	12 hr	24 hr	48 hr	2 hr	4 hr	6 hr	12 hr	24 hr	48 hr	2 hr	4 hr	6 hr	12 hr	24 hr	48 hr
Acetamiprid	0.1 g	63.39 (52.77)	66.73 (54.7)	79.99 (63.43)	81.78 (64.7)	100 (90)	100 (90)	0 (0)	19.33 (26.0)	26.53 (31)	55.53 (48.1)	55.53 (48.17)	74.20 (59.4)	0 (0)	10.02 (18.45)	20.01 (26.57)	32.98 (35.05)	36.94 (37.43)	44.41 (41.79)
Clothianidin	0.05 g	79.99 (63.43)	89.99 (71.5)	100 (90)	100 (90)	100 (90)	100 (90)	10.02 (18.4)	60 (50.7)	66.74 (54.7)	78.47 (62.3)	78.47 (62.35)	100 (90)	0 (0)	60.12 (50.84)	60.12 (50.84)	77.74 (61.85)	100 (90)	100 (90)
Imidacloprid	0.15 ml	59.99 (50.76)	73.47 (59)	83.64 (66.14)	94.70 (76.6)	98.46 (82.8)	100 (90)	10.02 (18.4)	30.01 (33.2)	50 (45)	51.83 (46.0)	51.83 (46.05)	78.47 (62.3)	10.02 (18.4)	30 (33.21)	49.99 (45)	55.52 (48.17)	70.48 (57.09)	98.46 (82.87)
Thiamethoxm	0.125 g	79.99 (63.43)	86.98 (68.8)	98.61 (83.23)	100 (90)	100 (90)	100 (90)	66.73 (54.7)	79.99 (63.4)	100 (90)	100 (90)	100 (90)	100 (90)	66.73 (54.7)	69.99 (56.78)	69.99 (56.78)	77.74 (61.85)	100 (90)	100 (90)
Chlorantraniliprole	0.15 ml	23.19 (28.79)	40 (39.2)	53.35 (46.92)	59.29 (50.3)	66.95 (54.9)	98.46 (82.8)	0 (0)	10.02 (18.4)	10.02 (18.4)	11.10 (19.4)	11.1 (19.46)	11.10 (19.4)	0 (0)	0 (0)	10.02 (18.45)	11.10 (19.46)	14.46 (22.35)	14.46 (22.35)
Chlorfenapyr	1 ml	0 (0)	10.02 (18.4)	40 (39.23)	44.41 (41.7)	66.63 (54.7)	78.46 (62.3)	0 (0)	13.03 (21.1)	36.60 (37.2)	44.32 (41.7)	48.14 (43.91)	55.62 (48.2)	0 (0.00)	10.02 (18.45)	30 (33.21)	44.41 (41.79)	62.99 (52.53)	62.99 (52.53)
Diafenthiuron	0.75 g	10 (18.45)	13.03 (21.1)	16.37 (23.87)	22.21 (28.1)	29.46 (32.8)	55.62 (48.2)	10.02 (18.4)	20.02 (26.5)	20.02 (26.5)	22.20 (28.1)	22.20 (28.11)	29.46 (32.8)	10.02 (18.4)	10.02 (18.45)	20.01 (26.57)	22.20 (28.11)	29.46 (32.87)	29.46 (32.87)
Spiromesifen	0.4 ml	4.76 (12.6)	10.02 (18.4)	13.03 (21.16)	14.46 (22.3)	22.21 (28.1)	44.41 (41.7)	10.02 (18.4)	10.02 (18.4)	10.02 (18.4)	14.46 (22.3)	14.46 (22.35)	22.20 (28.1)	0 (0)	0 (0)	10.02 (18.45)	14.46 (22.35)	22.21 (28.11)	22.21 (28.11)
Fipronil	1 ml	59.99 (50.76)	63.39 (52.7)	73.47 (59)	77.74 (61.8)	100 (90)	100 (90)	10.02 (18.4)	10.02 (18.4)	23.19 (28.7)	55.53 (48.1)	55.53 (48.17)	88.86 (70.5)	10.02 (18.4)	10.02 (18.45)	20.01 (26.57)	55.52 (48.17)	77.74 (61.85)	77.74 (61.85)
S.Em ±		2.19	1.75	2.68	2.58	2.57	2.85	0.67	1.69	1.41	2.30	2.06	2.26	0.67	1.13	1.13	1.63	1.80	2.52
C.D (0.05)		6.56	5.25	8.02	7.71	7.70	8.53	2.00	5.04	4.22	6.89	6.16	6.77	2.00	3.40	3.40	4.88	5.39	7.54

Figures in parentheses are angular transformed values
Mean followed by same alphabet do not differ significantly by DMRT (P = 0.05%)

REFERENCES

1. A.O.A.C. *Official Methods of Analysis*. Association of Official Analytical Chemists. (15th edn.), Washington, D.C. (1990).
2. Abbott, W.S., A method of computing the effectiveness of insecticides, *J. Econ. Entomol*. **18**: 265-267 (1925).
3. Aravind, J and Samiayyan, K., Safety evaluation of diafenthiuron 50WP to non target organisms, *Trends in Biosciences.* **7 (13):** 1434-1439 (2014).
4. Brittain, C., Bommarco, B., Vighi, M., Barmaz, S., Settele, J. and Potts, S.G. , Theimpact of an insecticide on insect flower visitation and pollination in an agricultural landscape. *Agric. For. Ent*. **12**: 259–266 (2010).
5. Gradish, A. E., Dupree, C. D., Shipp, S. L.,Harrisa C. R. and Fergusonc. G. Effect of reduced risk pesticides for use in greenhouse vegetable production on *Bombus impatiens* (Hymenoptera: Apidae). *Pest Manag. Sci*. **66:** 142–146 (2010).
6. Jeyalakshmi,T., Shanmugasundaram, R., Saravanan, M., Geetha, S., Sweatha,S., Mohan, S., Goparaju, A. and Balakrishna, M.P., Comparative toxicity of certain insecticides against *Apis cerana indica* under semi field and laboratory conditions. *Pestology*. **35(2):** 23 -28 (2011).
7. Kavi tha, J., Kuttalam S., Chandrasekaran, S. and Ramaraju K. Effect of spiromesifen 240SC on beneficial insects. *Ann Plant Protect Sci*. **14(2):** 343-345 (2006).
8. Kremen, C., Williams, N.M. and Tharp, R.W.. Crop pollination from native bees at risk from agricultural diversification. *Proceedings of the National Academy of Sciences*, USA. **99(26):** 16812-16816 (2002).
9. Pastagia, J.J. and Patel, M.B. Relative contact toxicity of some insecticides to worker bees of *Apis cerana* F. *Journal of Plant Protection and Environment*. **4(2):** 89-92 (2007).
10. Sharma, D and Abrol, D.P..Contact toxicity of some insecticides to honeybee *Apis mellifera* (L.) and *Apis cerana* (F.), *J Asia Pac Entomol*. **8(1):** 113-115 (2005).
11. Sokal, R.R and Rholf, F.J, *Biometry*. 2nd Edition. W.H. Freeman & Co., San Francisco1981.
12. Sundararaju, D. Occurrence of bee fauna and extent of pollination in insecticide sprayed ecosystem of cashew, *Journal of Palynology.* **39:** 121-125 (2003).
13. Vinothkumar, B., Kumaran, N., Boomathi, N., SaravananP.A and Kuttalam, S., Toxicity of spirotetramat 150 OD to honeybees, *Madras Agric. J*.. **97(1):** 86-87 (2010).