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Evaluating the Effects of Pesticides Used in East-Algerian Orchards on *Apis mellifera intermissa*: Enzymatic Activity of Acetylcholinesterase

D. Nabti, M. Achou and N. Soltani

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

Abstract: The widespread use of different pesticides in the agriculture generates negative effects in non-target organisms such as bees. These products which are widely used in Algeria threaten biodiversity. This paper aims at revealing the impact of the pesticides toxicity during two flowering periods (2011 and 2012) at two localities in eastern Algeria; one exposed (Ben Amar) in the county of El Taref and another considered as a control site (Sidi Kaci) in the same county. A biochemical analysis of the enzymatic activity of acetylcholinesterase (AChE) was conducted. An inhibition of AChE activity in bees collected from the site of Ben Amar has been found compared with those of Sidi Kaci. This confirms the presence of pollutants in the exposed site and therefore the effects of environmental stress. A significant difference in AChE activity in the same period of spring of the two years: 2011 and 2012 has been noticed.

Key words: Agriculture · Bees · Algeria · Toxicity · Stress

INTRODUCTION

Bees are the predominant and most important groups of pollinators in economic terms in many parts of the world. However, honeybees have been sorely tested in recent years, while at the same time, the number of agricultural crops depending on pollination has gradually increased [1, 2]. The problem of weak beehives in Algeria is raised acutely since the 1990s. The world of beekeeping is concerned about the health of bee colonies and the opportunities available to apply suitable medical treatments. The society is keenly interested in biodiversity and environmental quality in which the bees can be an indicator [3]. Pesticides are major threats to pollinators; these products contaminate bees via nectar and/or pollen [4]. These, can have direct consequences for the bees with honey and finally lead to the contamination of the honey, which becomes harmful for human health [5]. The acetylcholinesterase (AChE, EC 3.1.1.7.) represents a biomarker of neurotoxicity widely used for identifying exposure to anticholinesterase chemicals such as organophosphorous (OP) and carbamate (C) insecticides [6, 7]. Consequently, honeybee may be considered as a particularly pertinent model for the development of biomarkers to assess the environmental contamination.This work was devoted to evaluate the impact of the toxicity of pesticides used in orchards in the presence of beehive of *A. mellifera intermissa* during two periods of the year 2011-2012 at two locations. Identification of the toxicity of the used pesticidees has been tested by an enzymatic assay; the acetylcholinesterase (AChE).

MATERIALS AND METHODS

Biological Materials: Biological material represented by the Algerian bee *Apis mellifera intermissa* [8] which is the most widespread and its range extends along the North of Africa: Morocco, Tunisia and Algeria. It adapts the best with various habitats [9].The bees were collected monthly in spring of 2011 and 2012 from two sampling sites (Ben Amar, Sidi Kaci) and transferred to the laboratory.

Sampling and Identification of the Study Sites: Within the context of the study, the sampling process was carried out randomly on 10 beehives in the spring of 2011 and 2012 in two sites; one located in a forest area in

Corresponding Author: Djahida Nabti, Department of Biology, Faculty of Sciences,
Laboratory of Applied Animal Biology, Badji-Mokhtar University, Annaba, Algeria.

Table 1: The main pesticides used during the flowering period of 2011 and 2012

Commercial Pesticides	Chemicals Family	Active Ingredient	Months	Quantity/ha
Thiram 80%	Carbamate	Thiram	March (2011;2012)	2.5kg /ha
Topik 080 EC	Pyridylphenylethe	80g/L Clodinafoppropargil	March (2011)	4.5L/ha
Weedazol	Triazole	240g/L Aminotriazol And 250g/L Ammonium Thiocyanate	March (2011)	5L In 300L Water/ha
Dursban 4%	Organophosphate	480g/L Chlorpyrifos	April (2011)	1L In 600L Water
Trimangol 80%	Carbamate	Maneb	April (2011)	250g/ha
Lamdoc 50 EC	Synthetic Pyrethroid	50 Mg/L Lambda Cyhalothrin	May (2011)	1.25L/ha
Decis EC 25	Synthetic Pyrethroid	25g/L Deltamethrin	May (2011)	1L/ha
Marchal 25EC	Organophosphate	250g/L Carbosulfan.	April (2012)	2L/ha
Fenthion	Organophosphate	Fenthion	May (2011)	3.5L/ha
Cyper As 25 EC	Synthetic Pyrethroid	Cypermethrin	May (2012)	300ml/ha
Bulldock 025 SC And 70% Thiophanate-Methyl	Pyrethroid+ Carbamic Acid Derivative	25g/L Beta-Cyfluthrin And 70% Thiophanate-Methyl	June (2011;2012)	1L/ha; 2.5kg/ha
Karate+ Score 250 EC	Synthetic Pyrethroid	50g /L Lambda Cyhalothrin And 250g/L Difenoconazole	June (2011)	1.5L and1L/ha
70% Thiophanate-Methyl And Occidor 50 Sc	Carbamic Acid Derivative Carbamate	70% Thiophanate-Methyl And 500g/L Carbendazim	July (2011;2012)	(1.5kg+1L)/ha
Lamdok 50 EC	Synthetic Pyrethroid	50 Mg/L Lambda Cyhalothrin	July (2011)	1.5L/ha
Rotam (Rophosat 480)	Amino-Phosphonate	480g/L Glyphosate	July (2011)	8L/ha

Fig 1: Sampling of *A. mellifera intermissa*

Sidi kaci (county of El Taref 36° 45'N, 7° 58 'E) and the other exposed to pesticides in Ben Amar (36°47’29.8”N 7°48’53.9”E), with an area of 50 acres of fruit orchards. An investigation has been given to the farmers concerning the usage mode of pesticides, the dosage and the period of treatment (Table 1). The sampling of bees is done by hand at the entrance of the hives (Figure 1). About 50 bees have been sampled on every site.

Enzyme Essays: The *A. mellifera intermissa* heads were dissected during the same day and conserved in 1ml of detergent solution (38.03 mg Tris-ethylene glycolbeta- aminoethyl ether N NN 'N' or EGTA, 1 mL triton X 100%, 5.845 g NaCl, 80 ml ??;10 mMTris buffer) using an Ultrasonic homogenizer (Sonifer B-30) until biomarker analyses. Determination of AchE activity was Performed using a method described by [10] with the use of acetylthiocholine (ASCh) as substrate. The activity rate was measured as change in OD/min at 412 nm (ext.coeff. 13.6 Mm.cm). Activity was expressed as μmol/min/mg protein. The inhibition rate of the biomarker activity (%) was estimated by the following formula:

(Control site-Treated site)÷ Control site ×100

Statistical Analysis: Data are expressed by the means ± standard deviation (SD) and were subjected to two way analysis of variance (ANOVA). The comparison of mean values was carried out by Student’s t-test.

A significant difference was assumed when $p<0.05$ and $p\leq 0.001$. All statistical analyses were performed using MINITAB Software (Version 16, Penn State College, PA, USA).

RESULTS

Rate of Pesticides Used at the Treated Exploitation (Ben Amar) During the Spring of 2011 and 2012: Our farmers make, in average, 15 to 30 treatments per greenhouse tunnel throughout the growth cycle of a culture, with very dangerous synthetic products (Table 1). This figure shows their application percentage during the flowering period. A high rate (41.66%) of fungicides was observed during the spring of 2012 compared to 2011 and a reduced rate of insecticides (25%) compared to spring 2011.

Effect of Pesticides on the Enzymatic Activity of Ache in 2011: The enzymatic activity of AChE varied during the flowering period. A significant enzyme inhibition rate (44.39%) for samples of Ben Amar was noticed in May compared to those of March (29.66%) and April (42.51%), where a reduced activity has been witnessed. Statistical analysis of the mean values of AChE with the Student *t* test ($p\leq 0.001$), during which a minimum value of 1,34±0,15 µM/min/mg of protein at the exposed site was noticed compared to the bees of the control site which present a value of 2.41 ± 0.10 µM/min/mg of protein. ANOVA Statistical analysis with two classification criteria on the bees AchE activity from two localities (Sidi Kaci and Ben Amar) indicates the existence of very highly significant Site effect and time effect (Month) ($p\leq 0.001$) and no significant interaction site/Month ($P <0.05$), (Table 3).

Table 2: Comparison of pesticides' rates in Ben Amar during the same period of 2011 and 2012.

Pesticides	Insecticides	Fungicides	Herbicides	Acaricides
2011	37.50%	37.50%	18.75%	12.50%
2012	25%	41.66%	16.66%	18.18%

Table 3: Effect of pesticides used in orchards (kg/L) on the rate of AChE among workers of *A. mellifera intermissa* during spring 2011: Analysis of variance with two classification criteria (Sites / Month). *: Significant ($p\leq 0.05$); **: ($p\leq 0.01$) very significant; ***: highly significant ($p\leq 0.001$) : NS, no significant; P: Level of significance.

Sources	DDL	SCE	CM	Fobs	P
Sites	1	7.3856	7.3856	104.74	0.000***
Month	2	34.7010	17.3505	246.05	0.000***
Interaction Sites/month	2	0.3886	0.1943	2.76	0.104NS
Residual error	12	0.8462	0.0705		
Total	17	43.3214			

Table 4: Effect of pesticides used in orchards (kg/L) on the AChE rate among workers on *A. mellifera intermissa* in 2011: Analysis of variance with two classification criteria (Site/ Month).

Sources	DDL	SCE	CM	Fobs	P
Sites	1	7.0249	7.0249	26.34	0.000***
Month	2	1.5786	0.7893	2.96	0.090NS
Interaction Site/Month	2	0.4190	0.2095	0.79	0.478NS
Residual error	12	3.2002	0.2667		
Total	17	12.2227			

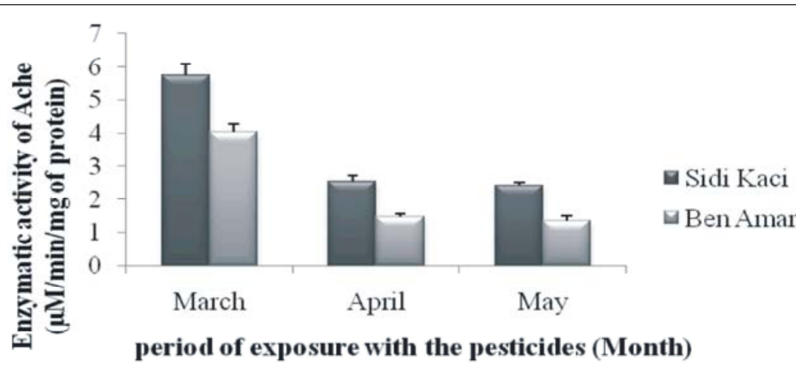

Fig 2: Effects of pesticides (kg/L) on the rate of AChE (µM/mg of protein) in *A. mellifera intermissa* bees during spring of 2011 (m ± s, n = 20).

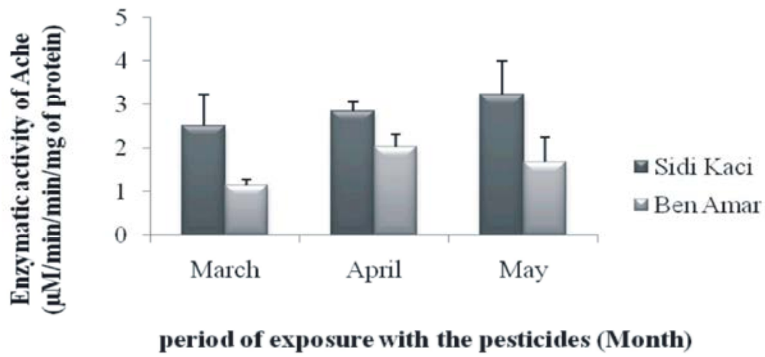

Fig 3: Effets of pesticides (kg/L) on the rate of AChE (µM/mg of proteins) in *A. mellifera intermissa* bees during spring of 2012 (m ± s, n = 5).

Effect of Pesticides on the Rate of Ache Activity in Workers of *A. Mellifera Intermissa* During 2012: Observations done during the three flowering periods show a fluctuation in the rate of the enzyme inhibition of AChE where 54.58% was noticed in March with a mean value of 2.51 ± 0.72 µM/min/mg protein in individuals of Sidi Kaci (control site) and 1.14 ± 0.13 µM / min/mg protein at the exposed site. The minimum AChE inhibition rates are recorded in the months of April (29.02%) and May (47.98%) with values of 2.03 ± 0.28 and 1.68 ± 0.57 µM/min/mg of protein for the site of Ben Amar respectively. Comparison of mean values by Student's *t* test revealed a highly significant decrease in the enzymatic activity of AChE during the flowering period of 2012 ($p \leq 0.01$). Statistical analysis of the results obtained by ANOVA 2 shows a very highly significant effect of pesticides used for the treatment of orchards on local field bees between the two study sites ($p \leq 0.001$). The time effect is also non-significant and similarly to the level of Site/Month interaction ($P < 0.05$), (Table 4).

Comparative Study of the Enzymatic Activity of the Ache Between the Flowering Period of 2011 and 2012: The enzymatic activity of the Ache has varied over the same study period between 2011 and 2012 at the treated site where a maximum value of 4.03 ± 0.26 µM/min/mg of protein was obtained during the month of March (2011) compared to that of 2012 which witnessed a low activity of 1.14±0.1326µM/min/mg of protein. However, the minimum of AChE rates for the year 2012 have been recorded in the first and last month of the study period with a value of 1.14±0.13 µM/min/m of protein in March and 1.68±0.57 µM/min/mg of protein in May. Statistical analysis by the Student's *t* test indicates a highly significant difference (p = 0.0013) in March, a significant difference in April (0.0342) and no significant differences were noticed during May (0.3767), (Table 5).

Table 5: Comparative study of the enzymatic activity of AChE in *A. mellifera intermissa* collected in Ben Amar for the spring period of 2011 and 2012 (n = 3, m ± sd).

	Month		
Year	March	April	May
2011	4.03±0.26	1.46±0.10	1.34±0.15
2012	1.14±0.13	2.03±0.28	1.68±0.57
P Value	0.0013	0.0342	0.3767

DISCUSSION

Environmental and agricultural welfare depend on a large number of different species of pollinators, including bees that contribute to the evolution of living beings and a large number of plants [11, 12]. The honeybee (*Apis mellifera L*) is an insect with a major role in agricultural production [13] and of another fields of health [14, 15]. For some years, their disappearances were frequently reported in several countries. This work is focused on the use of pesticides. These products can be toxic to bees on short-term, or, when used at low doses, lead to chronic effects that weaken or kill bees [16]. These negative consequences of using pesticides are described in detail in two recent reports by Greenpeace: The decline of bees [17] and Bees Bumblebee [18]. These contaminants are spreading throughout the treated plants and vegetables who are contaminated with pesticides residue [19], mainly in the pollen and the nectar [21, 22]. This contamination is a direct risk to the bees that collect these two nutrients and bring them into the hives; it causes poisoning symptoms such as hyper arousal, ataxia, convulsions, hypersensitivity, tremor and the paralysis and change in their behavior [23]. It is worth noting that toxicity and danger of active Ingredient against bees and fish (non-target organism) is reported in the datasheets of several products. Sefrazit, Thiram, Marchal and Cyper 25 EC are used during the flowering period with non-compliance with doses and the application frequencies. Ecotoxicological problems are not taken into account in the approval of pesticides in Algeria, despite evidence of effects on auxiliary insects such as inhibition of the enzymatic activity of AChE as shown in our study at the site of Ben Amar compared to control site (Sidi Kaci). Indeed a very highly significant difference $p \leq 0.001$ is noticed between the sites during the spring periods of 2011 and 2012. This inhibition is consistent with the results of Pallavi Srivastava and Ajay Singh. [24] Which revealed a 68% and 44% inhibition of the AchE activity in nervous tissues Freshwater Fish *Clarias batrachus* due to Trizole (Propiconazole). A similar inhibition of AChE was also reported in honey bee [25, 26].

In conclusion, our results indicate that the Honey bee (*A. mellifera intermissa)* can be used as a bioindicator for exposure to pesticides. Toxicity of these pesticides to animals is attributed to their ability to inhibit the acetylcholinesterase (AchE) enzyme from breaking down acetylcholine. As a result, changes and differences between the two sites in biomarker level may be related to the intensity and the duration of the stress detected at Ben Amar as compared to Sidi Kaci.

REFFERENCES

1. Kremen, C. and et A. miles, 2012. Ecosystem Services in Biologically Diversified versus Conventional Farming Systems: Benefits, Externalities and Trade-Offs. Ecology and Society, pp: 17.
2. Garibaldi, L.A., I. Steffan-Dewenter, R. Winfree, M.A. Aizen, R. Bommarco, S.A. Cunningham, C. Kremen, L.S.G. Carvalheiro, L.D. Harder, O. Afik, I. Bartomeus, Benjamin, F. Boreux, V. Cariveau, D. Chacoff, N.P. Dudenhöffer, J.H. Freitas, B.M.J. Ghazoul, S. Greenleaf, J. Hipólito, A. Holzschuh, B. Howlett, R. Isaacs, S.K. Javorek, Kennedy, C.M. Krewenka, K. Krishnan, S. Mandelik, Y. Mayfield, M.M. Motzke, I.T. Munyuli, B.A. Nault, M. Otieno, J.G. Petersen, S.G. Pisanty, Potts, Rader, R..T.H. Ricketts, M. Rundlof, C.L. Seymour, C. Schüepp, H. Szentgyörgyi, Taki, H. Tscharntke, T. Vergara, C.H. Viana, B.F. Wanger, T.C.C. Westphal, N. Williams and A.M. Klein, 2013. Wild Pollinators Enhance Fruit Set of Crops Regardless of Honey Bee Abundance. Science, 339: 1608-1611.
3. Directrion des statistiques agricoles (D.S.A), 2007.
4. Rortais, A., G. Arnold, M.P. Halm and F. Tou□et-Briens, 2005. Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by deferent categories of bees. Apidologie, 36: 71-83.
5. Lorenz, E.S., 2009. Potential health e?ects of pesticides, Ag Communications and Marketing, Wiley, New York, pp: 230-257.
6. Matozzo, V., A. Tomei and M.G. Marin, 2005. Acetylcholinesterase as abiomarker of exposure to neurotoxic compounds in the clam Tapes philippinarum from the Lagoon of Venice. Mar. Pollut. Bull., 50: 1686-1693.
7. Narbonne, J.F., N. Aarab, C. Clerandeau, M. Daubeze, J. Narbonne, O. Champeau and P. Garrigues, 2005. Scale of classification based on biochemical markers in mussels: Application to pollution monitoring in Mediterranean coasts and temporal trends. Biomarkers, 10: 58-71.
8. Buttel, R.H., 1906. Apistica. Beitrage zur Systematik, Biologie, Sowie zur geschichtlichen und geographischen Verbreitung der Honigbiene (Apis mellifera L.), ihrer Variet Aten und der iibrigen Apis-Arten. Veroff. Zool. Museum. Berlin, pp: 117-201.
9. Cornuet, J.M., A. Daoudi, H. Mohssine and J. Fresnaye,1988. Étude biométrique de population d'abeilles Marocaines. Apidologie., 19: 355-366.
10. Ellman, G.L., K.D. Courtney, V. Andres and R.M. Featherstone, 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol., 7: 88-95.
11. Gallai, N., J.M. Salles, J. Settele and B.E. Vaissiere, 2009. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecol. Econ., 68: 810-821.
12. Jayaramappa, K.V., Mahesh Pattabhiramaiah and H.R. Bhargava, 2011. Influence of Bee-attractants on Yield Parameters of Ridge Gourd (Luffa acutangula L.) (Cucurbitaceae), World Appl. Sci. J., 15(4): 457-462.
13. Kremen, C., N.M. Williams, M.A. aizen, B. Gemmillherren, G. LeBuhn, R. minckley, L. Packer, S.G. Potts, Ta roulston, I. steffan-dewenter, D.P. Vazquez, R. Winfree, L adams, E.E. Crone, S.S. Greenleaf, T.H. Keitt, A.M. Klein, J. regetz and T.H. et Ricketts, 2007. Pollination and other ecosystem services produced by mobile organisms: a conceptual framework for the effects of landuse change. Ecology Letters., 10: 299-314.

14. Khaled El-Menabbawy, Suzette I. Helal, H.M. Iman R. and Ahmad Gaffar Hegazi, 2014. Possible Role of Bee Venom Therapy in Children with Cerebral Palsy. World J. Med. Sci., 11(1): 27-32.
15. Eswaran, V.U. and H.R. Bhargava, 2014. Chemical Analysis and Anti-Microbial Activity of Karnataka Bee Bread of Apis species, World Appl. Sci. J., 32(3): 379-385.
16. Krupke, C.H., G.J. Hunt, B.D. Eitzer, G. Andino and K. Given, 2012. Multiple Routes of Pesticide Exposure for Honey Bees Living Near Agricultural Fields, Plos One 7(1): e29268. doi:10.1371/journal.pone.0029268.
17. Tirado, R., G. Simon and P. Johnston, 2013. Le déclin des abeilles-analyse des facteurs qui mettent en péril les pollinisateurs et l'agriculture en Europe. Laboratoires de recherche de Greenpeace. Rapport technique 01-2013.http: //www. greenpeace. org/france/ PageFiles/266577/ Le%20 declin% 20des%20abeilles_20130425_BD.pdf.
18. Johnston, P., C. Huxdorff, G. Simon and D. Santillo, 2014. Les abeilles ont le bourdon : Analyse des résidus de pesticides retrouvés dans le pain d'abeille et le pollen piégé d'abeilles domestiques (Apis mellifera) dans 12 pays européens. Laboratoires de recherche de Greenpeace, rapport technique 03-2014. http://www.greenpeace.org/france/Global/france/openspace/Greenpeace_Pollen_Final. pdf.
19. Subhash Chandra, Anil N. Mahindrakar and L.P. Shinde, 2012. Capillary Gas Chromatography-Mass Spectrometry Determination of Pesticide Residues in Vegetables. Middle-East J. Sci. Res., 11(5): 589-594.
20. Saijad Ahmed Baig, Nizar Ahmed Akhtera, Muhammad Ashfag and Muhammad Rafique Asi 2009. Determination of the Organophosphorus Pesticides in Vegetables by High-Perfomance liquid Chromatographiy. American-Eurasian J. Agric. and Environ. Sci., 6(5): 513-519.
21. Bernal, J., E. Garrido-Bailon, M.J. del Nozal, A.V. Gonzalez-Porto, R. Martin-Hernandez, J.C. Diego, J.J. Jimenez, J.L. Bernal and M. et Higes, 2010. Overview of pesticide residues in stored pollen and their potential effect on bee colony (*Apis mellifera*) losses in Spain. Journal of Economic Entomology, 103: 1964-1971.
22. Dively, G.P. and A. et Kamel, 2012. Insecticide residues in pollen and nectar of a cucurbit crop and their potential exposure to pollinators. J. Agr. Food. Chem., 60: 4449-4456.
23. Aliouane, Y., A.K. El hassani, V. Gary, C. armengaud, M. Lambin and M. Gauthier, 2009. Subchronic exposure of honeybees to sublethal doses of pesticides: Effects on behavior. Environ. Toxicol. Chem., 28: 113-122.
24. Pallavi, S. and A. Singh, 2014. Behavioral Changes by Inhibition of Acetylcholinesterase Induced by Trizole (Propiconazole) Fungicide on Freshwater Fish Clarias batrachus. World J. Fish & Marine Sci, 6(1): 82-86.
25. Westlake, G.E., A.R. Hardy and J.H. Stevenson, 1985. Effects of Storage and Pesticide Treatments on Honey Bee Brain Acetyl Cholinesterase Activities. Bull. Environ Contam. Toxicol., 34: 668-675.
26. Dulin, F., M.P.H. Lemeille, S. Lozano, A. Lepailleur, J. Sopkova-de Oliveira Santos, S. Rault and R. Bureau, 2012. Interpretation of honeybees contact toxicity associated to acetylcholinesterase inhibitors, Ecotoxicol. Environ. Safety., 79: 13-21.