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The toxic effect of the pesticides on *Apis mellifera intermissa (Hymenoptera, Apidae): Glutathione S-Transferase Activity*

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ABSTRACT

The aim of the present study is to evaluate the impact of pesticides used in orchards of Annaba region (North-East Algeria) on Apis mellifera intermissa, during the spring of 2011. The control site was represented by a region located in Sidi Kaci (Wilaye El Taref) forest while the second site was represented by an orchard fruits located in Ben Amar (Wilaye El Taref) which was treated with various pesticides. Traps were placed near and under the hives placed in fields in order to estimate the mortality of honey bees for each site. The results revealed a high mortality in treated site compared to the control site. The pesticide toxicity was confirmed by the GST measurement activity. The results showed a significant induction on specific activity of GST in local bees.

Keywords: *Apis mellifera intermissa*, Wilaya El Taref, Pesticides, Mortality, GST.

INTRODUCTION

Honey bees are a vital part of our agricultural system. It plays an important role in global food production [1]. Currently bee-keeping and the systems agricultural and ecological which depend on the action on pollinating, live one critical moment with respect to the reduction with large scales of the populations of bees. This degradation result from multiple, factors such as pathogens, bad nutrition, natural habitat degradation, and pesticides [2-3] .but one conspicuous threat is their unintended exposure to agricultural pesticides that protect crops from pest. Their negative consequences are described in detail in two reports published recently by Greenpeace: The decline of bees [4] and the bees have bumblebee [5] .The present study indicate that the intensive use of pesticides including organophosphates, carbamates, pyrethroids, and neonicotinoids in Algerian orchards. Algeria is one of the countries that use large amounts of conventional pesticides in agriculture within over than 400 phytosanitary products have been registered including 40 varieties that are widely used by agricultors [6] .The intensification of agriculture can starve bees [4] , with effects potentially prejudicial for the bees which need to find a balance nutritional optimal to guarantee their growth and their reproduction [7]. Biochemical biomarkers are increasingly used in ecological risk assessments of aquatic and terrestrial ecosystems to identify the incidence of exposure to and effects caused by xenobiotics. These contaminants induce biotransforming enzymes that catalyze phase I or II reactions [8-9]. While glutathione S-transferases (GST) play a key role in the cellular detoxification and excretion of a variety of xenobiotic compounds in bacteria, plants, and animals[10-11]. The aim of this study was to use GST biomarkers to investigate the influence of site quality on health status of a local population of *Apis mellifera intermisa*. This insect has been previously used as a sentinel species in environmental assessment[12] .

MATERIALS AND METHODS

Presentation of sampling sites

In this study, sampling of bees was conducted randomly on a five hives for each of the two study sites during the spring of 2011. The treated site is represented an orchard situated in the agglomeration of Ben Amar (Wilaya El

taref): (Figure 1.2) with a latitude 36°46'31.15"N.and longitude of 7°48'07.66"E,that is recognized as agricultural zones, therefore it is exposed to various phytosanitary products. Another site that located in the forest zone of Sidi Kaci (Wilaya El taref): (Figure 3) with a latitude of 36° 45'N, 7° 58' E. The traps were placed under the hives in order to collect the dead bees. For the bioassays, only the spring season was selected because of flowering period. The Bees were manually picked up from the hive entrance, 20 individuals were sampled at each site once a month. To sacrifice bees, they were placed in freezer. The evaluation of pesticides toxicity was performed in the laboratory by measuring the enzyme activity of detoxification biomarker Glutathion-S- Transferase.

Fig. 1: Disposition of the hives in Treated Site (Ben Amar)

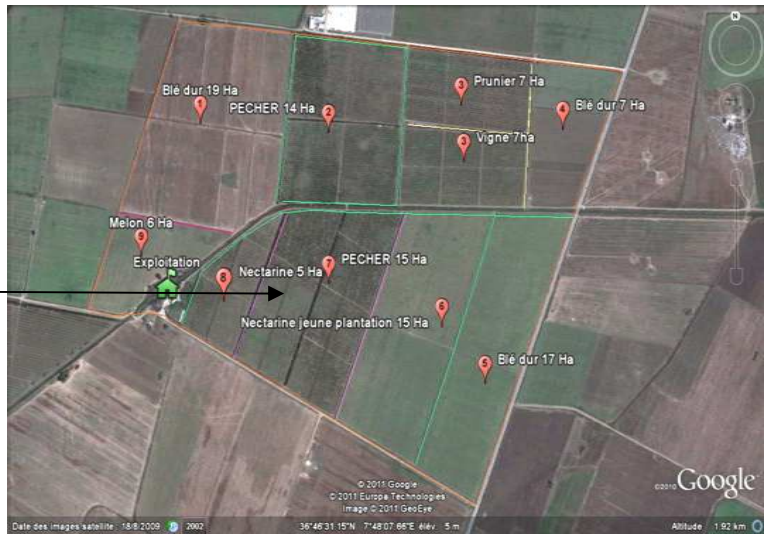

Fig .2: Distribution of the orchards

Fig. 3: Disposition of the hives in control site (Sidi Kaci)

Pesticides used

Topik 080 EC, Weedazol ,Thiram 80%, Dursban 5G, Fastac 100EC, OPUS ,Captan ,Omite 57E, Trimangol 80%, Lamdoc 50 EC, Decis EC 25, Aliette flash ,Ortiva, Mispilan

Samples collection

The bees of *A mellifera intermisssa* [13] were collected monthly in spring 2011, from two sampling sites (Ben Amar ,Sidi Kaci) and transferred to the laboratory.

Enzyme essays

GST activity was measured using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate in a final reaction mixture containing 1 mM CDNB and 5 mM reduced glutathione [14]. The activity rate was measured as change in optical density (OD/min) at 340 nm (ext. coeff. 9.6 mM. cm-1) and the final activity was expressed as µmol/min/mg protein. The protein content was evaluated [15] using serum albumin as standard (BSA, Sigma).

Statistical analysis

Data are expressed by the means ± standard deviation (SD) and were subjected to two way analysis of variance (ANOVA). The comparison of means values was made by Student's t-test. A significant difference was assumed when $p < 0.05$. $p \leq 0.001$.All statistical analyses were performed using MINITAB Software (Version 16, Penn State College, PA, USA).

RESULTS

Mortality of *A. mellifera intermissa* in Spring 2011

The Statistical analysis ANOVA 1 of the monthly mortality in two localities (Sidi Kassi and Ben Amar) indicates a very significant difference $p \leq 0.01$ between the two studies sites in hive 4 and significant difference in the others hives ($p \leq 0.05$).

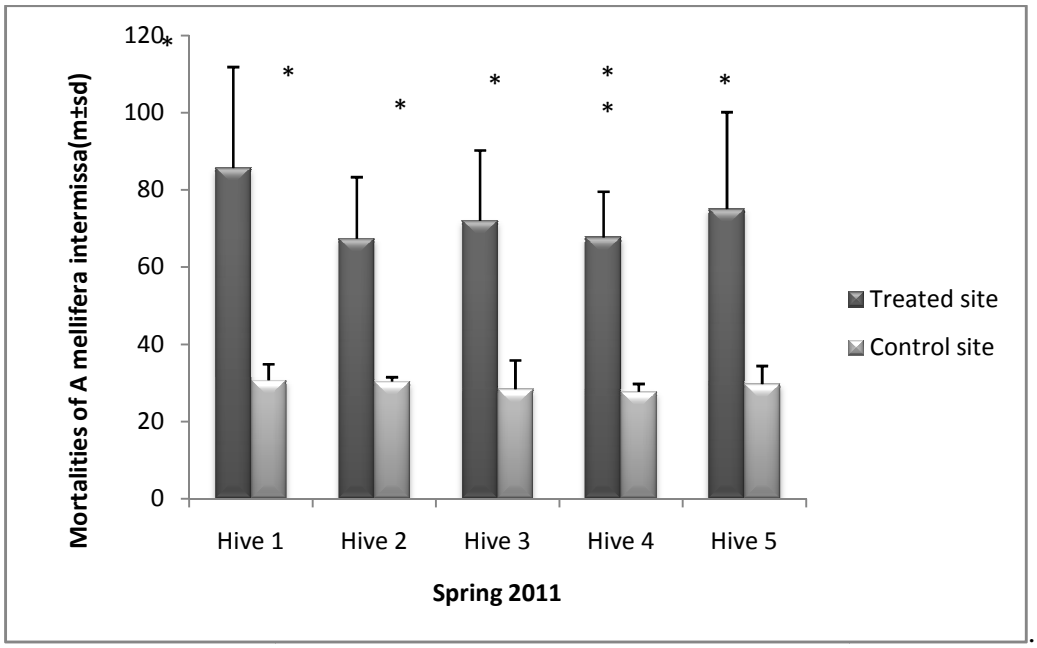

Fig. 4: Mortality of *Apis mellifera intermissa* recorded in the two sites during the spring 2011 (n=5).*: significant ($p \leq 0.05$); **: ($p \leq 0.01$) very significant; *: highly significant ($p \leq 0.001$). *P*: Level of significance**

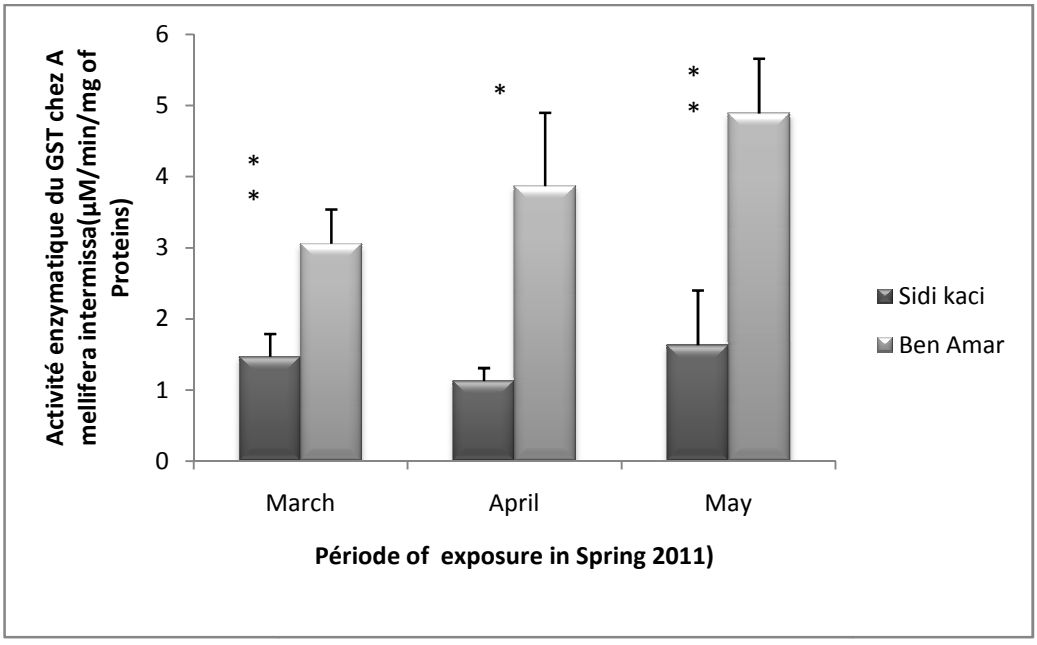

Fig. 5: The specific activity of glutathione S-transferase (µM/min/mg of proteins) in A. *mellifera intermissa* from two sites of Annaba gulf during spring 2011 (mean ± SD; n= 5)

Specific activity of GST

Data on GST measurements are presented in Table 1 and figure 2. The comparison of the averages by the test "T" of Student revealed an induction very significant of the GST ($p \leq 0.01$) at the individuals of Ben Amar during the spring of 2011. For March (P=0.009), April (P=0.011), May (P=0.007).

The analysis of the variance with two criteria of classification indicates that there' is a highly significant sites effect ($p \leq 0.001$), no time effect (P=0.058), and a non significant interaction Sites/Month ($p>0.05$): (Table1).

Table 1: The specific activity of GST (µM/mn/mg of protein) in *A.mellifera intermissa* from two sites of El Taref gulf: Two-way ANOVA

Sources	DDL	SCE	CM	Fobs	P
Sites	1	28.8547	28.8547	65.35	0.000***
Month	2	3.2223	1.6112	3.65	0.058 NS
Interaction Sites/Month	2	2.1951	1.0976	2.49	0.125 NS
Residual Error	12	5.2988	0.4416		
Total	17	39.571			

DISCUSSION

Domestic bees are the most distributed species which constitute the main group of pollinating and are the most important economical insect in many areas of the world. Unfortunately in last decade, a high mortality of honey bee, *Apis mellifera intermissa*, was frequently reported in countries in Africa. In Algeria, during the last few years the mortality that varies between 11% and 90%, of honey bees was declared by farmers mainly in agricultural regions [16]. Honey bee colony losses are greater now than any time in recent history [17-18]. According to a recent report of the European Authority of Safety of the Food [19], knowledge on the multiple factors of stresses which touch the pollinating savages and servants are very insufficient on a European scale, in particular on the effects harmful of the cocktails of pesticides. All the insecticides of which the use in treatment of the seeds, is propagated in the whole of the treated plant, in particular in pollen and E nectar [20-21]. This contamination thus poses a direct risk for the bees which collect these two nutritive elements and bring back them in the hives. Biomarkers have been extensively used to reveal the exposure of organisms to various chemicals in the environment. They are based on physiological, biochemical, anatomical and behavioral parameters the perturbation of which persists after the exposure to the pesticide [22] .The induction of GST activity has been used as a biomarker of exposure to xenobiotics, that catalyze the conjugation of variety of electrophilic substrate to the thiol group of GSH, producing less toxic forms [23].It play a key role in the cellular detoxification and excretion of a variety of xenobiotic compounds in bacteria, plants, and animals[10-11] . Specific activity of GST (in µmol/min/mg protein) increased to 4.89± 0.77at May ($p \leq 0.01$), 3.87±1.03 at April ($p \leq 0.05$) and 3.06± 0.48 at March ($p \leq 0.01$).Significant effects ($p < 0.001$) of site and interaction sites/months) no significant were determined by ANOVA tow-way test. In fact, GST activity of *A. mellifera intermissa* increased upon pesticides exposure, coinciding with the study of [24] sowed that some Acaricides increase the GST activity in the larval, pupae and nurse bees of *Apis mellifera intermissa* as compared to controls, [25] demonstrate also that the pollutants increase GST activity of digestive gland at the bivalves of *Mya arenaria*. The studies former studies former to the laboratory of animal biology applied showed a significant increase ($p < 0.05$) in GST activity as compared with controls in the marine bivalve Donax trunculus L. (Mollusca, Bivalvia) [26].

CONCLUSION

This paper reports the results on detoxification enzyme (GST) measured in the *A. mellifera intermissa* an useful sentinel for environmental. The result revealed an important rate on the level of Ben Amar as compared to Sidi Kaci level may be related to the intensity used of the pesticides in orchards site.

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