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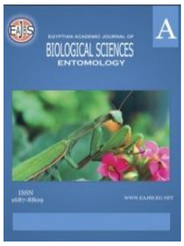

Variation in Oral Acute Toxicity of Thiamethoxam According to the Volume Administered in Algerian Honeybees

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ABSTRACT

Thiamethoxam is a neurotoxic systemic insecticide belonging to the neonicotinoid family. Approved under the trade Algeria Actara WG 25%, the plant protection product is recognized as toxic to the bees after acute exposure. However, this product is persistent, has a significant residual activity and accumulates in plants. It is therefore necessary to completely reassess its toxicity. To do this, we determined the sensitivity of the Saharan and Tellian honeybee, *Apis mellifera sahariensis* and *Apis mellifera intermissa*, to thiamethoxam by testing the acute oral toxicity on worker bees in the laboratory. The study is based on determining the LD_{50} according to the volume administrated. Each batch of bees was fed 100, 200, 500 and 1000µl (5, 10, 25 and 50µl per bee) 55,5% (w/v) sucrose solution with increasing doses (1, 10, 20, 50, 70 and 90 ng per bee) of thiamethoxam dissolved in acetone, for trial treatments, and 55,5% (w/v) sucrose solution supplemented with acetone, control treatments. The results showed that the toxicity is manifested by acute symptoms of early neurotoxicity and cumulative mortalities that occur 24 hours after treatment. The LD_{50} varies with the volume administered. Indeed, the LD_{50} values decrease with increasing volume, and there is an inverse relationship between the LD_{50} obtained and administered volumes.

INTRODUCTION

The honeybee, *Apis mellifera*, is widely recognized as an insect of great agronomic, ecological, and scientific importance as well as an important test model for pesticide effects on other insect pollinators (Suchail *et al*., 2001; Saleem *et al*, 2020). It contributes to more than 80% of the total pollination in agriculture and plays an important role in pollination in ecosystems (Breeze *et al*., 2011, Willmer *et al*., 2017). However, beekeepers are seeing serious weaknesses in their colonies and highlight the responsibility for certain insecticides used in plant protection. Currently, these impairments are known as CDD (colony collapse

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disorder). This is an unexplained phenomenon that is characterized by a sudden disappearance of bees from the hive in the Northern Hemisphere (Oldroyd, 2007). These losses are reported in the United States (Ellis et Leconte, 2008; 2010; Ellis *et al*., 2010; Van Engelsdorp *et al.,* 2010), Canada (Currie *et al.*, 2010) and Japan (Neumann et Carreck, 2010). The same is noted in northern Europe as in Norway (Dahle, 2010), Poland (Topolska *et al*., 2010), Scotland (Gray *et al*., 2010), Denmark (Vejsnaes et Kryger, 2010), England (Aston, 2010) and the Netherlands (Van Der Zee, 2010). Some countries in the European center are affected by this phenomenon, notably Switzerland (Charriere et Neumann, 2010) and Austria (Brodschneider *et al*., 2010). Neither is the Mediterranean region spared, represented by France (Chauzat *et al*., 2010a), Italy (Mutinelli *et al*., 2010), Bosnia and Herzegovina (Santrac *et al*., 2010), Bulgaria (Ivanova et Petrov, 2010), Croatia (Gajger *et al*., 2010) and Greece (Gallina et Mutinelli , 2010 ; Hatjina *et al*., 2010). Multiple causes of CCD (Colony Collapse Disorder) are discerned. Biotic factors include pathogens, parasites and insufficient food resources due to fragmentation and habitat loss. The abiotic factors involved in the same process are climate change and pollutants (Decourtye *et al*., 2010).

Pesticides are frequently identified as responsible for falls in populations of *Apis mellifera* Linné, 1758 (Greig-Smith *et al*., 1994 ; Lefebvre et Bruneau, 2003 ; Barnett *et al*., 2007 ; Chauzat *et al*., 2010b ; Johnson *et al*., 2010 ; Medrzycki *et al.,* 2010 ; Marzaro *et al*.,2011 ; Belzunces *et al*., 2012, Tavares *et al*.,2017 ; Saleem *et al,* 2020). In Algeria, this problem has been reported in recent years, with a worsening of poisoning phenomena in bees (Chahbar *et al.*, 2011, 2014, 2018). Indeed, many beekeepers report a weakening and/or total depopulation of the colony. This may be due to changes in the nervous system of bees because 90% of the insecticides used in the field have neurotoxic properties. The Actara 25% WG end-use product containing Thiamethoxam is known to be toxic to bees and is banned from use during the flowering period. However, bees may be exposed to thiamethoxam and other neonicotinoids during these foraging trips of the hive to collect floral resources, water and resins, thereby increasing the risk of exposure to lethal and sublethal levels. Our work consists of determining the sensitivity of the local honey bee *Apis mellifera intermissa* and *Apis mellifera sahariensis* by testing the acute oral toxicity of thiamethoxam which gives indications on the threshold of sublethally. The study is based on the determination of LD_{50} based on volumes administered.

MATERIALS AND METHODS

Material:

Thiamethoxam is marketed under the brand Actara for foliar and soil and Cruiser seed treatment (Maienfisch *et al*., 2001). It is 99.7% purity and was obtained from the office Syngenta Algeria. For each subspecies, bee workers (*A. m. intermissa* and *A. m. Sahariensis*) were captured from honey and pollen combs in the same healthy queen-right colony for all bioassays; all drones were discarded. Immediately before treatment, bees were anesthetized with carbon dioxide and kept in cages (10,5 x 7,5 x 11,5cm) in a temperature-controlled chamber at 25±2°C with 60 ±10% relative humidity. Bees were fed with candy and water ad libitum (EPPO, 1992).

Experimental Conditions:

In each experiment, three cages of 20 bees were used for each dose of treatment. Experiments were replicated at least three times; control mortality was less than 15% in all experiments (EPPO, 1993).

Modes of Treatment:

Oral Application:

The honeybees were deprived of food for 2h before administration of thiamethoxam. Thiamethoxam solutions were prepared in a 1% acetone solution and then diluted 10-fold in the 50% (w/v) feeding sucrose solution. The final concentration of acetone solution in the sucrose solutions of control and assay tests was 0.1% (v/v). The dosing solutions were prepared fresh for each test. Each lot of bee is fed with 100, 200, 500 and 1000 µL (5, 10, 25 and 50 µL per bee) of sucrose solution 55,5% (w / v) final with 5 increasing doses (1ng , 10ng, 20ng, 50ng, 70 ng and 90 ng per bee) of active substances (thiamethoxam) dissolved in acetone for the test treatments, and of 55.5% (w / v) final sucrose solution supplemented with acetone for control treatments. After consuming this solution, bees were fed with candy and water ad libitum. Mortality was recorded at 24, 48 and 72 h.

Data Analysis:

Mortality data were corrected according to Abbott (1925). The LD_{50} values are obtained by probit transformation of mortality rates. One-way analysis of variance was used to evaluate differences between groups.

RESULTS AND DISCUSSION

The dose-related mortality curves for the different volumes administered in honey bees *A. m. intermissa* and *A. m. sahariensis* (Fig. 1) show that the higher the dose, the higher the mortality. Therefore, there is a directly proportional relationship between the dose of thiamethoxam administered and the observed mortality. In addition, it should be noted that the mortality changes over time until reaching its maximum for each dose after 24 hours. 5µl containing the highest dose of 90 ng ingested by the bee causes mortality rates of 98.4% (*A.m. intermissa*) and 93.3% (*A.m. sahariensis*). On the other hand, the dose of 1ng / bee causes very low mortality percentages of the order of 1.7% (*A.m. intermissa*) and 3.3% (*A.m. sahariensis*). Similarly, the ingestion of 10 µl of contaminated solution at a dose of 90 ng per bee results in 100% mortality rates whereas the low dose of 1 ng/bee is accompanied by low mortality rates of 1.7%. (*A.m. intermissa*) and 0% (*A. m. sahariensis*). The same high dose present in 25 and 50 µl of contaminated solution administered by bees still produces very high mortality rates exceeding 90% for both breeds of bees. The low dose of 1 ng/bee causes mortality percentages of between 20% and 43.4% for *A. m. intermissa* and between 18.4% and 25% for *A. m. sahariensis*. In fact, it should be noted that the more the volume ingested by the bee exceeds 10µl, the lower the doses result in high mortality rates. Analysis of ANOVA variance ($p < 0.0001$) indicates a significant difference in thiamethoxam sensitivity in *A. m. intermissa* and at *A. m. sahariensis* after oral application regardless of the volume ingested.

LD_{50} of Thiamethoxam

The LD_{50} variations obtained from the volumes administered for the two races of bees *A. m. intermissa* and *A. m. sahariensis* are shown in Table 1.

Table 1: Variation in LD_{50} Values versus Volumes Administered in *A. m.intermissa* and *A. m. sahariensis*.

	Oral LD_{50} (ng / bee)							
	A.m. intermissa				***A.m. sahariensis***			
	5µl	10µl	25µl	50µl	5µl	10µl	25µl	50µl
24h	19,7	11,89	6	1,98	31,51	11,47	10,02	7,15
48h	19,7	11,89	5,77	1,58	30,93	10,86	9,74	6,25
72h	14,2	11,89	5,67	1,54	30,49	10,86	8,57	6,11

For *A. m. intermissa*, the LD_{50} obtained vary according to the volumes administered. Indeed, for volume 5µl, the LD_{50} is 19.7 ng/bee both after 24h and 48h, and 14.2 ng / bee after 72h (Table 1). For a volume of 10µl, the LD_{50} is 11.9 ng/bee at the different observation times. For higher volumes, 25 and 50 µl per bee (Table 1), the LD_{50} is less than 10 ng/bee at different observation times. It is the same for *A. m. sahariensis*, the LD_{50} is close to 31.5 ng for a volume of 5µl and 11.4 ng / bee for a volume of 10 µl per bee. However, this LD_{50} is less than 10 ng/bee at 25 and 50 µl volumes. It appears that the values of the LD_{50} decrease with increasing volume. Thus, there is an inversely proportional relationship between the LD_{50} obtained and the volumes administered. It should be noted that the LD_{50} for the volume of 5µl / bee is 10 times greater than the LD_{50} obtained with a volume of 50 µl/ bee. The LD_{50} obtained for the Saharan bee are generally higher than the LD_{50} obtained in the Tellian bee. These variations of LD_{50} between the two races are significant. From these results, it appears that *A. m. sahariensis* is less sensitive than *A. m. intermissa* but the toxicity of thiamethoxam is extremely high.

Thiamethoxam induces the same symptoms of neurotoxicity whatever the volume ingested, symptoms of poisoning similar to those observed with other neonicotinoid insecticides are noticed (Bortolotti *et al*., 2003. Medrzicky *et al*., 2003 ; Maccagnani *et al*., 2008 ; Chahbar *et al.,* 2011 et 2014). It appears that the values of the LD_{50} decrease with increasing volume. It should be noted that there is an inversely proportional relationship between the LD_{50} obtained and the volumes administered. Indeed, the LD_{50} for the volume of 5 µl / bee is 10 times greater than the LD_{50} obtained with a volume of 50 µl / bee in *A. m. intermissa*. For *A. m. sahariensis*, the LD_{50} obtained with a volume of 5 µl / bee is 5 times greater than the LD_{50} observed with a volume of 50 µl / bee. The same findings are observed by Madouni et Oudni (2010). The LD_{50} obtained for the Saharan bee are in general stronger than the LD_{50} noted in the Tellian bee. This difference in susceptibility of bees to thiamethoxam cannot be due to experimental errors because all experiments are performed in a standardized way according to method 95 of the Commission of biological tests (C.E.B., 1995). This difference in susceptibility between colonies may be due to the variation in the detoxification capacity of thiamethoxam by bees. Indeed, in most cases, when a xenobiotic (substance foreign to the organism considered) enters an organism, the latter tries to eliminate it by changing its molecular structure to make it more soluble and easier to eliminate. This metabolism generally leads to the formation of less toxic metabolites. But it can also in some cases lead to the formation of toxic or even more toxic metabolites than the parent product, such as clothianidin, which is more toxic than thaimethoxam itself. (Nauen *et al*., 2003; Ford et Casida, 2006, 2008; Kamakar *et al*., 2009; Benzidane *et al*., 2010; Casida, 2011; Zhou *et al*., 2012). The low doses contained in the volumes of 5 µl and 10 µl, cause low mortality rates. It is to be noted that when the bee ingests beyond 10 µl of solution at low doses mortality rates appear high. Thiamethoxam induced mortality at low doses may be due to the lack of induction of detoxification enzymes. Low doses would not be sufficient to trigger the induction of detoxification enzymes. The induced enzymes cause the increase in the rate of metabolism and the excretion of thiamethoxam. At higher doses, the increase in mortality is due to the induction of detoxification enzymes that result in the formation of more toxic metabolites such as clothianidin. In the case of imidacloprid, Suchail (2001) hypothesizes that the increase in high dose mortality may be due to the saturation of detoxification enzymes and that the induction of detoxification enzymes necessitates the formation of metabolites less toxic than the parent product.

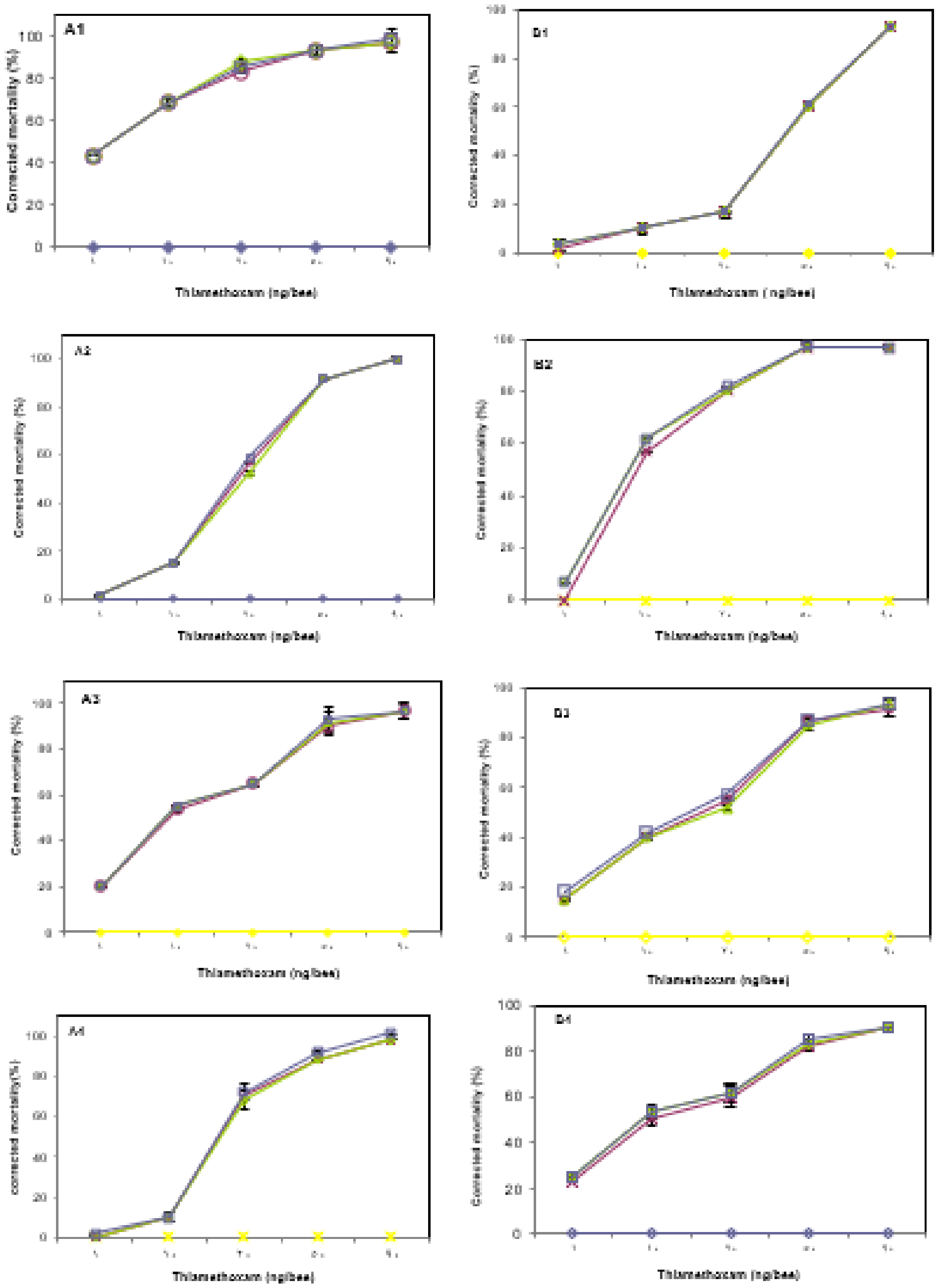

Fig. 1 (A1, A2, A3, A4, B1, B2, B3 & B4). Dose-response relation resulting from oral exposure to Thiamethoxam. (A) *Apis mellifera intermissa*. (B) *Apis mellifera sahariensis*. Bee mortality was observed 24h (x), 48h (◊) and 72 h (□) after oral application of different thiamethoxam doses. Data represented the means ± SD of three experiments performed in triplicate. The absence of error bars corresponds to SD = 0.

CONCLUSION

The study of the variation of the acute toxicity of thiamethoxam as a function of the volumes ingested made it possible to highlight important characteristics.

The first characteristic is that the toxicity of thiamethoxam increases with the volumes ingested. Whatever the breed of bee, this toxicity of thiamethoxam is extremely high. These results confirm the danger associated with this insecticide which should not be used in full bloom, to limit the risk of bee poisoning.

The second characteristic is the rapid onset of symptoms of neurotoxicity and the mortality that occurs only after 1 hour to 5 hours depending on the volume ingested

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ARABIC SUMMARY

الاختلاف في السمية الحادة الفموية لثيامثوكسام حسب الحجم المعطى لدى نحل العسل الجزائري

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ثياميثوكسام (Thiamethoxam) مبيد حشري جهازي عصبي ينتمي إلى عائلة النيونيكوتينويد. تم إقراره كمنتج وقاية النبات في الجزائر تحت الاسم التجاري أكتارا بنسبة 25%، وتم التعرف عليه كمنتج سام للنحل بعد التعرض الحاد له. ومع ذلك، يبقى مستمر الفعالية، مخلفاته نشطة ويتراكم في النباتات. لذلك من الضروري مراجعة سميته كليتا. قمنا في المخبر بتحديد حساسية نحل العسل الصحراوي والتلي *Apis melifera sahariensis & Apis melifera intermissa*، إلى thiamethoxam من خلال اختبار السمية الفموية الحادة على النحل العامل. تعتمد هذه الدراسة على تحديد LD_{50} وفقًا للحجم المعطى. بالنسبة للمعالجات التجريبية، كل مجموعة من النحل تمت تغذيتها بـ 100 و200 و500 و1000 مايكرولتر من محلول سكروز 55.5٪ (وزن / حجم)، ما يعادل (5، 10، 25، 50 ميكرولتر لكل نحلة)، وبجرعات متزايدة (1، 10، 20، 50، 70 و90 نانوغرام لكل نحلة) من الثيامثوكسام المذاب في الأسيتون. وبالنسبة لمعالجات المراقبة تمت بـ 55.5٪ (وزن / حجم) محلول سكروز مكمل بالأسيتون. بينت النتائج أن السمية تظهر من خلال الأعراض الحادة للتسمم العصبي المبكر، ومعدل الوفيات التراكمي يحدث بعد 24 ساعة من المعالجة. تختلف LD_{50} باختلاف الحجم المعطى. في الواقع، تنخفض قيم LD_{50} مع زيادة الحجم المعطى، وهناك علاقة عكسية بين LD_{50} التي تم الحصول عليها والكميات المعطاة.