Article

Toxicity of a botanical insecticide on *Bombus impatiens*, *Apis mellifera*, *Chrysoperla carnea* and *Orius insidiosus*

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Abstract

The indiscriminate use of synthetic insecticides increases the risks of contamination to the environment, damage to health and reduction of populations of beneficial organisms, as well as selection of resistance of pests to these products. Given this scenario, efficient and less harmful alternatives are required for humans and non-target organisms. In this research the acute toxicity of botanical insecticide-acaricide BIODIe® (based on plant extracts of castor bean, chicalote and berberis) was evaluated on two pollinators -bees and bumblebees- and on the predators *Chrysoperla carnea* and *Orius insidiosus*, three methodologies were used of exposure: direct contact, residual contact and oral toxicity. The botanical insecticide was slightly toxic to the pollinators and predators studied, and was classified in category 1 of the international biological control organization (IOBC) due to low mortality (<25%). This suggests that this product represents low risks for non-target organisms in their implementation in integrated pest management.

Keywords: *Chrysoperla carnea*, *Orius insidiosus*, biorational insecticides, pollinators.

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Introduction

Insecticides based on botanical extracts are used to control a wide variety of pests and represent an alternative to replace or reduce applications with organosynthetic insecticides (Baldin *et al*. 2007; Descamps *et al*., 2008; Ateyyat *et al*., 2009; Franca *et al*., 2009; Perales *et al*., 2015). The principle of these insecticides is based on the ability of some plants to produce toxicity, repellency or hinder the growth of pest organisms, they can also affect their feeding or discourage oviposition, besides presenting low toxicity on mammals (Reddy and Guerrero, 2004; Stefanazzi *et al*., 2006; Bleeker *et al*., 2009) and degrade faster in the environment (Schmutterer, 1990).

Several investigations suggest the importance in the selection of the product based on the type of pest and crop, in addition to the impact on natural enemies and pollinators (Horowitz *et al*., 2009, Gonzalez-Maldonado, 2012). The latter are a relevant group in agroecosystems (Gallai *et al*., 2009) and natural ecosystems (Kwak *et al*., 1998) pollinators of greater economic importance are bees and bumblebees, an estimated 75% of fruit production, vegetables and seeds depend on the pollination of these hymenoptera (Cutler *et al*., 2014), so 35% of the world's agricultural and food production requires them (Klein *et al*., 2007), which represents an annual global value of 153 billion euros (Gallai *et al*., 2009).

Despite the importance of pollinators, the impact of human actions has reduced 45% of bee populations in the United States alone, during the last 60 years (NAS, 2007). Most of these losses, between 1966 and 1979, were attributed to the use of organochlorine insecticides, carbamates, organophosphates and pyrethroids (Atkins and Kellum, 1986). Although chemical pesticides play an important role in modern agriculture, they are not always compatible with pollinators, parasitoids or agricultural pest predators since they are usually more sensitive to intoxication than target pests (Johansen, 1977).

The danger of pesticide poisoning for bees and entomophagous insects is not only by direct contact, but also by the intake of contaminated nectar, pollen, and water, which carry and can affect the hive (Thomazoni *et al*., 2009). Due to the presumably low toxicity in mammals of some botanical insecticides (Stefanazzi *et al*., 2006; Bleeker *et al*., 2009), as well as their more rapid degradation in the environment (Schmutterer, 1990), the effects of some of those products on non-target organisms and consider their possible inclusion in pest management proposals. Therefore, the objective of this research was to determine the acute toxicity of a botanical insecticide to two pollinators, *Bombus impatiens* Cresson and *Apis mellifera* L. and the predators *Chrysoperla carnea* (Stephens) and *Orius insidiosus* (Say).

Materials and methods

The research was carried out in the biological control laboratory of the Postgraduate College campus Montecillo, Texcoco, State of Mexico. The bees (*A. mellifera*) were workers in active foraging period (approximately 2-3 weeks of age) obtained from a colony established on the same campus. Bumblebees (*B. impatiens*) were 2 to 5 days old and were purchased from a commercial brood in Queretaro, Mexico (Koppert, Mexico).

The predators *O. insidiosus* and *C. carnea* were acquired from the companies -Koppert México and Organismos Beneficos para la Agricultura, SA de CV, respectively. For the experiments, adults of *O. insidiosus* and larvae III of *C. carnea* were used, since they are the stages of development that are commonly used in biological control programs.

Insecticides and exposure methods

Biodie® insecticide belongs to the chemical group of carboxyl, has insecticide-acaricide application and is composed of four compounds: argemonin (Chicalote, *Argemone mexicana* L.), berberine (berberis, *Berberis* sp.) ricinine (castor bean, *Ricinus communis* L.) and α-Terthienyl. This product was evaluated on the four species, at a dose of 5mL L^{-1} of water, through three methods of exposure: direct contact, residual contact and oral toxicity (contaminated food). In the first two methods, the Potter tower was used, incorporating in both Inex-A (1 mL L^{-1}). In the exposure of contaminated food insecticide was mixed with honey (50:50) and provided in a jar with a cotton wick. The amount of insecticide varied depending on the type of application and in all cases, there was a control with application of distilled water. Each treatment and control had five replications; All exposed organisms were kept in bioclimatic chamber (25 ±2 °C, 60% RH and 12:12 L: O), fed with water and honey and observations were made in the intervals of 4, 24, 48, 72 and 96 h. A dead insect was considered to be the individual who did not present movement or who had erratic flight and no opportunity to perform his normal activities.

Bees and bumblebees followed the procedures issued for evaluation on pollinators issued by the United States Environmental Protection Agency's (USEPA), with the exception of using 20 insects as an experimental unit, with a tolerated mortality in the witness of 10%, instead of 25 organisms and 20% mortality. The weight of these hymenoptera was recorded before and after the application (USEPA, 2012). For predators, recognized methodologies were made for predatory insects (Hassan, 1994; 2009; Viñuela *et al*., 2001; Schneider *et al*., 2003; Rimoldi *et al*., 2008; Fogel *et al*., 2009), using as an experimental unit 10 individuals of each species by repetition.

Toxicity by direct contact

The bioassays with pollinators were made based on the guide 'Ecological Effects Test Guidelines OCSPP 850.3020: Honey Bee Acute Contact Toxicity Test' (USEPA, 2012), Hymenoptera were sedated with CO_2 and kept in a glass Petri dish (θ= 15 cm), with a filter paper disc in the base. 3 mL of the solution, insecticide-distilled-adherent water, was applied at 25 lbs of pressure. The response variable was mortality after insecticide application. The larvae of *C. carnea* were subjected to 3 °C for 15 min to immobilize them and then they were placed in glass Petri dishes (θ= 9 cm) with a disc of filter paper at their base. The insecticide was applied at a pressure of 20 lb and each larva was isolated, to avoid cannibalism, in containers (θ= 2.5 cm x a= 1.2 cm) with organza mesh on the lid to favor ventilation. In *O. insidiosus* the same methodology was followed with the variant of keeping 10 adults (3-4 days) per Petri dish (θ= 5 cm x a= 1.5 cm), each with a cotton wick to provide water mixed with honey to 10%. Both species were fed *at libitum* with eggs of *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae).

Residual contact toxicity

It used the methodology described in 'Ecological Effects Test Guidelines OCSPP 850.3030: Honey Bee Toxicity of Residues on Foliage' proposed by the United States Environmental Protection Agency's (USEPA, 2012), with some modifications. The Hymenoptera were anesthetized with CO_2, for 10 s, then they were introduced in the Petri dishes previously contaminated with 10 mL of the solution. In the case of predators, test tubes (θ= 1 cm x a= 10 cm) impregnated with 1 mL of the insecticide solution were used, which were shaken manually for 20 s and then the remaining solution was removed and kept at room temperature. environment for 1 h for the evaporation of the waste. Subsequently, the previously anesthetized insects were introduced. The lacewings were individually placed by tube to avoid cannibalism while the hemipterans were introduced in groups of 10 individuals.

Oral toxicity

The procedure described in the Guidelines for the testing of chemicals, Honeybees acute oral toxicity test (USEPA, 2012), was used, with some modifications. The insects of this experiment were fasted for 2 h in plastic containers of 25x14x13 cm. The insecticide was prepared at a dose of 5 mL of the product dissolved in 1 L of water with honey (50:50), this mixture was offered for 3 h and subsequently it was replaced by food without contaminants. In the case of predators, *S. cerealella* eggs were first contaminated, which were adhered to plastic strips (0.7 cm by 0.5 cm) with commercial white glue and immersed for 10 s in the insecticide solution, leaving for a period of time at room temperature to evaporate the excess and placed in Petri dishes (θ= 2.5 cm x a= 1.2 cm). The predators, with previous fasting of 2 h, were placed in these containers. The larvae of *C. carnea* were introduced individually per container and the hemipterans were placed in groups of 10 individuals.

Statistic analysis

In all cases, the number of dead insects was compared between treatments at 4, 24, 48, 72 and 96 h after insecticide application; due to the fact that the data did not meet the normality assumptions, a signed Wilcoxon rank test was used to compare the mortalities between the treatment against the control. In the case of pollinators, their weight was recorded before and after the application of insecticides and compared by a t-student test. All the analyzes were carried out with the statistical program SPSS.

Results and discussion

The hymenopterans evaluated showed little weight variation *intra* taxon, the lower weight of bees compared to bumblebees is considered an intrinsic issue to the species. There were no differences between the weight of each group of insects by type of treatment in relation to the control (Table 1).

Table 1. Weight of bees and bumblebees used in the bioassays in three methods of exposure to Biodie®.

Treatment	Average weight (g ± EE)					
	Direct contact		Residual contact		Contaminated food	
	Bees	Bumblebees	Bees	Bumblebees	Bees	Bumblebees
Biodie®	0.034 ±0.001	0.059 ±0.004	0.031 ±0.002	0.044 ±0.001	0.028 ±0.001	0.038 ±0.001
Control	0.033 ±0.001	0.056 ±0.004	0.028 ±0.001	0.044 ±0.001	0.031 ±0.001	0.039 ±0.001
Statistics	t = 1.15 *p*= 0.88	t= 0.36 *p*= 0.72	t= 1.15 *p*= 0.27	t= 0.2 *p*= 0.84	t= 1.68 *p*= 0.13	t= 0.15 *p*= 0.88

EE= standard error; in all experiments n= 20.

In the bumblebees there was no mortality in any form of exposure to the plant extract (Biodie®) during the evaluation times. While in the bees there was mortality in all three forms of exposure, registering a gradual increase with higher values in the period 72 to 96 h in the methods of direct contact and contamination of the food. However, none exceeded 25% mortality, suggested by the international biological control organization (IOBC) as the maximum limit for cataloging the product in the category “slightly toxic” on beneficial insects (Hassan, 1994). The control did not surpass 7% of mortality in any of the cases (Table 2), this mortality was attributed, in part, to the flight and shock inside the experimental vessels and probably due to carrying out the bioassay in bright conditions, since it is recommended that these types of experiments are kept in darkness. The experiment was designed in this way to ensure greater activity of the bees, and an experimental condition closer to what could actually happen.

Table 2. Percentage of mortality in bees in bioassays by different methods of exposure.

Type of exposure/treatment	Percentage of mortality (average ± SE)		
	48 h	72 h	96 h
Direct contact			
Biodie®	2.5 ±0.83	10.5 ±2.03	21.5 ±3.41
Control	1 ±0.66	4 ±1	7 ±1.5
Statistics*	W^+= 90, p= 0.28	W^+= 76, p= 0.03	W^+ = 68.5, $p\leq$ 0.005
Residual contact			
Biodie®	13 ±1.5	19 ±2.2	23 ±2
Control	1.5 ±0.76	5 ±1	7 ±1.3
Statistics*	W^+ = 58, $p\leq$ 0.001	W^+ = 60, $p\leq$ 0.001	W^+ = 56, $p\leq$ 0.001
Food contamination			
Biodie®	3.5 ±2.1	11.5 ±3.5	23.5 ±3.1
Control	0.5 ±0.5	5.0 ±1	8.5 ±1
Statistics*	W^+ = 94, p= 0.44	W^+ = 90, p= 0.28	W^+ = 59, $p\leq$ 0.001

*= at 4 and 24 h the mortality was zero or very low, therefore it does not appear in the table. W^+= value of the statistic of the test of the ranges with sign of Wilcoxon.

The mortality of lacewings larvae did not exceed 11% in the control or in the treatments, so it is suggested that there is tolerance to the insecticide in the three methodologies that were evaluated (Table 3). Adults of *O. insidiosus* exposed to the insecticide had mortality ≤13% at any time of observation and treatment. Mortality in these hemipterans started after 24 h, registering its highest value at 48 h without variations in the following evaluations. Only the direct contact treatment differed with respect to the control, although it did not exceed 9% (Table 4).

Table 3. Percentage of mortality in larvae of *Chrysoperla carnea* at different times after being exposed to Biodie® in three application methods.

Type of exposure/treatment	Percentage of mortality (average ± SE)			
	24 h	48 h	72 h	96 h
Direct contact				
Biodie®	3 ±3	3 ±3	3 ±3	3 ±3
Control	4 ±1.6	4 ±1.6	4 ± 1.6	4 ±1.6
Statistics*	W^+= 85, p= 0.14	W^+= 92, p= 0.35	W^+= 92, p= 0.35	W^+= 92, p= 0.35
Residual contact				
Biodie®	9 ±3.1	10 ± 3	11 ±2.8	11 ±2.8
Control	1 ±1	1 ± 1	1 ±1	1 ±1
Statistics	W^+= 79, p= 0.05	W^+= 74, p= 0.02	W^+= 69, p= 0.005	W^+= 69, p= 0.005
Contaminated food				
Biodie®	5 ±1.7	7 ±1.5	8 ±1.3	8 ±1.3
Control	2 ±1.3	4 ±2.2	4 ±2.2	4 ±2.2
Statistics	W^+= 97.5, p= 0.58	W^+= 93, p= 0.39	W^+= 93, p= 0.39	W^+= 93, p= 0.39

*= at 4 and 24 h the mortality was zero or very low, therefore it does not appear in the table. W^+= value of the statistic of the test of the ranges with sign of Wilcoxon.

Table 4. Percentage of adult mortality of *Orius insidiosus* at different times after being exposed to Biodie® in three different methods of exposure.

Type of exhibition/treatment	Percentage of mortality (average ± standard error)			
	24 h	48 h	72 h	96 h
Direct contact				
Biodie®	6 ±2.2	9 ±2.3	9 ±2.3	9 ±2.3
Control	2 ±1.3	2 ±1.3	2 ±1.3	2 ±1.3
Statistics	W^+= 89, p= 0.25	W^+= 78, p= 0.04	W^+= 78, p= 0.04	W^+= 78, p= 0.04
Residual contact				
Biodie®	3 ±1.5	4 ±1.6	4 ±1.6	4 ±1.6
Control	1 ±1	1 ±1	1 ±1	1 ±1
Statistics	W^+= 95, p= 0.49	W^+= 90, p= 0.28	W^+= 90, p= 0.28	W^+= 90, p= 0.28
Contaminated food				
Biodie®	5 ±2.2	13 ±3.7	13 ±3.7	13 ±3.7
Control	3 ±1.5	3 ±1.5	3 ±1.5	3 ±1.5
Statistics	W^+= 98.5, p= 0.63	W^+= 79, p= 0.05	W^+= 79, p= 0.05	W^+= 79, p= 0.05

*= at 4 and 24 h the mortality was zero or very low, therefore it does not appear in the table. W^+= value of the statistic of the test of the ranges with sign of Wilcoxon.

Discussion

In Europe and North America, the use of botanical pesticides began 150 years before the appearance of synthetic pesticides (organochlorines, organophosphates, carbamates and pyrethroids). However, synthetic products quickly relegated to those of botanical origin despite the possible negative effects to their use (Perry *et al*., 1998; Johnson *et al*., 2010). Currently, some botanical extracts may represent a possible alternative to synthetic pesticides; however, toxicity tests are needed on non-target organisms and not only on insect pests (Römbke *et al*., 2006). The main utility of using non-target organisms as bioindicators is to show the effects of the toxic at the individual level, and thus infer the effects at the population level (Iannacone and Alvariño, 2005).

According to the classification of toxicity of insecticides on natural enemies for laboratory tests, according to the IOBC (Hassan, 1992), the results of the present work are included within the first category of toxicity. (1= inoffensive, <30% mortality, the IOBC (Hassan, 1992) suggests that if an insecticide is found that is not toxic to a particular natural enemy in the laboratory, it is likely that it is not toxic to the same insect in the field, Therefore, no additional tests of semi-field or field will be necessary. For the case of *C. carnea* larvae, several investigations have documented resistance to a considerable number of organosynthetic insecticides, among them cypermethrin and deltamethrin (Ishaaya and Casida, 1981), phosmet and carbaryl (Grafton and Hoy, 1986), in addition to diazinon (Hoy, 1994) Some aqueous extracts of pyru (*Schinus molle*) and lantana (*Lantana camara*) are reported inoffensive for larvae of the first instar of *C. carnea* (Iannacone and Lamas, 2003).

While the agricultural soap and azadirachtin had no harmful residual effects on adults of *O. insidiosus* (Oetting and Latimer, 1995), being in category 1 of the IOBC. A botanical insecticide based on *Chenopodium* was evaluated on this same species of hemiptera, registering a mortality of 26% in 48 h, and decreasing at 72 h according to the recommended dose of 5 g ia. per L of water (Bostanian *et al*., 2005). With the information generated in this work, it is possible to indicate that there is a low risk when using the Biodie® insecticide, based on vegetal extracts on third instar lacewings and adults of pirate bugs.

As indicated above, in bumblebees there was no mortality in any form of exposure to the Biodie® product. While in bees it did exist in all three forms of exposure, but mortality was never exceeded by 25%, so it was established as a 'slightly toxic' insecticide, according to the IOBC classification (Hassan, 1994). Among the possible explanations for this difference between species, the size and weight of each individual must be pointed out, the bumblebees always had a greater weight than the bees; therefore, the probabilities of reaching lethal doses of some product will always be different; On the other hand, different susceptibility to products of botanical origin in different species has also been demonstrated (Iannacone and Lamas, 2003).

It is important to indicate that in this work only direct mortality was evaluated; with some organosynthetic insecticides it has been demonstrated that the exposure of bees to sublethal doses has serious consequences, including the interruption of their capacity to communicate the location of the sources of nectar and pollen, the depletion of the bees, reduction of their life cycles, mutations in offspring, weight loss and suppression of the immune system (Smirle, 1984; Schneider *et al*., 2009; Sánchez *et al*., 2016).

Such effects should be considered in future work with botanical insecticides because some work suggests that bees can be used successfully in flowering crops that have been treated with neem extract, probably due to their lower permanence in the environment (Melatophoulus *et al*., 2000) or because botanical products have not demonstrated the systemic effect of many organosynthetic insecticides. From the social and economic point of view, the identification of active compounds as potential candidates for the development of new phytosanitary products will provide effective and environmentally safe alternatives for agriculture (Pérez, 2012), for this reason the importance of continuing with this type of evaluations. with products of botanical origin.

Conclusions

The botanical insecticide was slightly toxic for the pollinators and predators studied, and was classified in category 1 of the international biological control organization (IOBC), due to the low mortality (<25%). This suggests that this product represents low risks for non-target organisms (pollinators and predators) in their implementation in integrated pest management.

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