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# Chemical control of Africanized Honey Bees (*Apis mellifera*) from the perspective of public health surveillance

# Control químico de las abejas melíferas africanizadas (*Apis mellifera*) en perspectiva de la salud pública

# Controle químico de abelhas africanizadas (*Apis mellifera*) na perspectiva de vigilância em saúde pública

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***ABSTRACT***
***Objective:*** *to compare the time of the knockdown effect between the pesticides prallethrin and dichlorvos on Africanized Honey bees (AHB).* ***Method:*** *this is an experimental study. The effect of these two pesticides over time was evaluated based on a life table methodology. The probability of survival between the two groups was compared based on the Mantel-Cox test.* ***Results:*** *it was observed that within 40 seconds the estimated probability of survival (no knockdown) was approximately 20% for prallethrin and 70% for dichlorvos. Estimated probability of survival (no knockdown) for prallethrin was lower in comparison to dichlorvos up to approximately 90 seconds. After that, the estimated survival for both pesticides became closer and stable. After 300 seconds almost all AHB were eliminated by both*

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*pesticides. Prallethrin showed better effect to control AHB when compared to dichlorvos.* ***Conclusion:*** *based on the results and considering that prallethrin presents lower toxicity to humans and to the environment it can be a safer alternative rather than dichlorvos to control AHB in urban areas.*
***Descriptors:*** *Public Health Surveillance; Bees; Animals, Poisonous; Insect Control; Insecticides.*

***RESUMEN***
***Objetivo:*** *comparar el tiempo del efecto knockdown de los pesticidas praletrina y diclorvos en las abejas melíferas africanizadas (AMA).* ***Método:*** *este es un estudio experimental. El efecto de estos dos pesticidas se evaluó con base en una metodología life table. La probabilidad de supervivencia entre los dos grupos se comparó con base en la prueba de Mantel-Cox.* ***Resultados:*** *a los 40 segundos la probabilidad estimada de supervivencia (sin knockdown) bajo el efecto de praletrina fue aproximadamente del 20% y diclorvos fue aproximadamente del 70%. La probabilidad estimada de supervivencia a praletrina fue menor hasta aproximadamente 90 segundos. Después de eso, la supervivencia estimada para ambos pesticidas se volvió más cercana y estable. Después de 300 segundos, casi todos las AMA fueron eliminados para ambos pesticidas. La praletrina mostró un mejor efecto para controlar las AMA en comparación con el diclorvos.* ***Conclusión:*** *la praletrina presenta una toxicidad más baja para los humanos y para el medio ambiente y puede ser una alternativa más segura que el diclorvos para controlar las AMA en áreas urbanas.*
***Descriptores:*** *Vigilancia en Salud Pública; Abejas; Animales Venenosos; Control de Insectos; Insecticidas.*

**RESUMO**
**Objetivo:** comparar o tempo do efeito knockdown entre os pesticidas praletrina e diclorvos em abelhas africanizadas. **Método:** trata-se de um estudo experimental. O efeito dos pesticidas foi avaliado pela metodologia *life table*. A probabilidade de sobrevivência entre os dois grupos foi comparada pelo teste de Mantel-Cox. **Resultados**: foi observado no tempo de 40 segundos que a probabilidade estimada de sobrevida (sem knockdown) para a praletrina era de aproximadamente 20% e para diclorvos de aproximadamente 70%. A probabilidade estimada de sobrevida das abelhas africanizadas sob efeito da praletrina foi menor quando comparada ao diclorvos até aproximadamente 90 segundos. Depois disso, a sobrevivência estimada para ambos os pesticidas se tornaram mais próximas e estáveis. Após 300 segundos praticamente todos foram eliminados com ambos os pesticidas. A praletrina apresentou melhor eficácia no controle das abelhas africanizadas quando comparada ao diclorvos. **Conclusão:** com base nos resultados e considerando que a praletrina apresenta menor toxicidade para humanos e meio ambiente, ela parece ser uma alternativa mais segura do que os diclorvos para o controle de abelhas africanizadas em áreas urbanas.
**Descritores:** Vigilância em Saúde Pública; Abelhas; Animais Venenosos; Controle de Insetos; Inseticidas.

## *INTRODUCTION*

*In Brazil, São Paulo city answered 95,607 requests to control bees and wasps between 2006 and 2015, an average of 9,560 requests per year. Among the demands received for eusocial bee species, there were interventions only for Apis mellifera.*

*Brazilian native eusocial bees do not have sting and therefore they are unable to cause accidents*[1], except some species from genus bombus[2]. *However, in the early 19th century the European subspecies Apis mellifera mellifera which does have sting, was introduced in Brazil which has aggravated by the process of Africanization verified in the 1950s, due to the introduction of Apis mellifera scutellata from Africa. Hybrids between European and African bees have spread along the Americas. They have begun to demand attention regarding to aggressiveness and potential risk to public health*[3-7].

*The high aggressiveness of these colonies and their close contact with humans in urban areas has resulted in a high number of stinging accidents involving serious medical consequences*[5,8,9].

*In urban areas, most Africanized bees live in artificial constructions. One study in São Paulo*[10,11] *showed that the periods of the year characterized by high temperatures and low rainfall have strong influence in the activity of bees and in the number of wandering swarms. Boxes, fences, garages, garbage and roofs are potential nesting sites for hives and swarms.*

*In Brazil, bee stings represented 6% of poisoning cases and 9% of deaths due to animal poisoning. Most of the cases involving bee stings are asymptomatic or mild, accounting for around 90% of the notifications, while moderate poisoning accounts for 10-18%, severe for 0.8-1.3% and the rate of fatality is from 0.3 to 0.4%*[9,12].

*According to the Brazilian Ministry of Health, the number of accidents involving these insects reached over 91,165 between 2006 and 2015 in Brazil leading to 288 deaths*[9]. *Since 1994 a program to control bees and wasps has been created in urban areas of São Paulo City to avoid accidents*[13,14].

*The first step to control bees or wasps is to correctly identify the species and its nesting sites. In some cases, according to a risk analysis, the nest may be controlled by using pesticide.*

*In this sense the use of a pesticide need to be highly efficient to knockdown the Africanized Honeybees (AHB), avoiding undesirable contamination and attack of insects. Knockdown may be defined as the state of intoxication and partial paralysis which usually precedes death of the insect[15].*

*Since 1990s, Dichlorvos (DDVP) has been used in the control of AHB in São Paulo city. This pesticide is an organophosphate widely used to control pests[16].*

*Organophosphates reduce acetylcholinesterase activity. Thus, the organ becomes overestimulated by an excess of acetylcholine, the impulse-transmitting substance responsible in the nerve ending that lead the animal to knockdown and death in general[17]. Additionally there is a large amount of information in the literature related to the risks associated to exposures to dichlorvos on humans and other species[16-20].*

*As an alternative, prallethrin is a pyrethroid widely used due to its high effectiveness and low toxicity for humans and the environment[21,22].*

*Pyrethroids alter the normal function of insect nerves by modifying the kinetics of voltage-sensitive sodium channels, which mediate the transient increase in the sodium permeability of the nerve membrane that underlies the rising phase of the nerve action potential[20]. Pyrethroids are 2,250 times more toxic to insects than to mammals because insects have increased sodium channel sensitivity, smaller body size, and lower body temperature[21].*

*Both organophosphates and pyrethroids work by disrupting the insect's nervous system[22]. According to the Globally Harmonised Classification System (GSH) dichlorvos (5 GSH classification entries) is more hazardous than prallethrin (4 GSH classification entries)[23,24].*

*Based on the above discussion, a comparative study was carried out to evaluate* *knockdown effects by comparison between prallethrin and dichlorvos on AHB.*

## *METHOD*

*This is an experimental study. A similar technique was used on demands to control AHB in urban areas of São Paulo City. However, an adaptation of the technique had to be made to measure knockdown effects on AHB.*

*Containers of Polyethylene terephthalate, diameter 120mm, height 208mm and volume of 2.5 $m^3$ were used. A hole on the cover of approximately 2.5 cm in diameter was done to apply the spray insecticide. The AHB was collected directly from a hive and introduced into the containers in order to avoid stress of the bees. The hive was found in a cavity of a wall, in north zone of São Paulo City. The trial was performed on light cycle under the following climatic conditions: temperature between 20 to 23ºC and humidity between 50 to 68%.*

*It was used 45 containers in a total of 15 repetitions. Each trial was performed with three containers: one received prallethrin, one dichlorvos and the third one was used as a control in order to check for knockdown and mortality by stress. There were no death and knockdown on the control group. Thus, only the containers receiving pesticides were considered in the statistical analysis. All AHB in the control group were released after each trial. The knockdown time for 334 AHB was recorded (averaging 10 bees per container); 167 bees on dichlorvos effect (10mg.$mL^{-1}$ active ingredient) and 167 bees on prallethrin effect (0,25mg. $mL^{-1}$ active ingredient). The amount of pesticide used was fixed in 15mL per container, for each product.*

*To verify the efficacy of pesticides on AHB, the number of bees that suffered knockdown was registered in the following time intervals: 0 |- 40 sec; 40 | - 60 sec; 60 | - 90 sec; 90 | - 120 sec; 120 | - 180 sec; 180 | - 300 sec and 300 | - 600 sec.*

*The effect of these two pesticides over time was evaluated based on a life table methodology, adequate for actuarially collected* *data[25]. The probability of survival between the two groups was compared based on the Mantel-Cox test[26,27]. In all cases, a p-value less than 0.05 indicated statistical difference.*

## RESULTS

*After application of pesticides, the amount of AHB in knockdown was counted at the pre-set time intervals. Using the life table methodology [25] it was possible to evaluate the effect of the pesticides over time and to estimate the probability of survival (no knockdown) at each time interval (Tables 1 and 2).*

*From the data in tables 1 and 2 it is possible to perform a comparative analysis of the efficacy of two pesticides (Figure 1).*

***Table 1 - Prallethrin: lifetime estimates for the probability of survival (no knockdown). 22/06/2017 to 06/04/2018. São Paulo-SP, Brazil.***

| Time (second) | Start | Knockdown within range | Estimated probability of survival (no knockdown) | Standard error | Confidence Interval 95% |
|---|---|---|---|---|---|
| 0 --40 | 167 | 133 | 1.0000 | -- | - |
| 40 --60 | 34 | 10 | 0.2036 | 0.0312 | (0.1424, 0.2648) |
| 60 --90 | 24 | 10 | 0.1437 | 0.0271 | (0.0906, 0.1968) |
| 90 --120 | 14 | 4 | 0.0838 | 0.0214 | (0.0419, 0.1257) |
| 120 --180 | 10 | 7 | 0.0599 | 0.0184 | (0.0238, 0.0960) |
| 180 --300 | 3 | 2 | 0.0180 | 0.0103 | (0.0000, 0.0382) |
| 300 --600 | 1 | 1 | 0.0060 | 0.0060 | (0.0000, 0.0178) |

***Table 2 - Dichlorvos: lifetime estimates for the probability of survival (no knockdown). 22/06/2017 to 06/04/2018. São Paulo-SP, Brazil.***

| Time (second) | Start | Knockdown within range | Estimated probability of survival (No knockdown) | Standard error | Confidence Interval 95% |
|---|---|---|---|---|---|
| 0 --40 | 167 | 53 | 1.0000 | -- | -- |
| 40 --60 | 114 | 65 | 0.6826 | 0.0360 | (0.6120, 0.7532) |
| 60 --90 | 49 | 38 | 0.2934 | 0.0352 | (0.2244, 0.3624) |
| 90 --120 | 11 | 8 | 0.0659 | 0.0192 | (0.0283, 0.1035) |
| 120 --180 | 3 | 3 | 0.0180 | 0.0103 | (0.0000, 0.0382) |
| 180 --300 | -- | -- | -- | -- | -- |
| 300 --600 | -- | -- | -- | -- | -- |

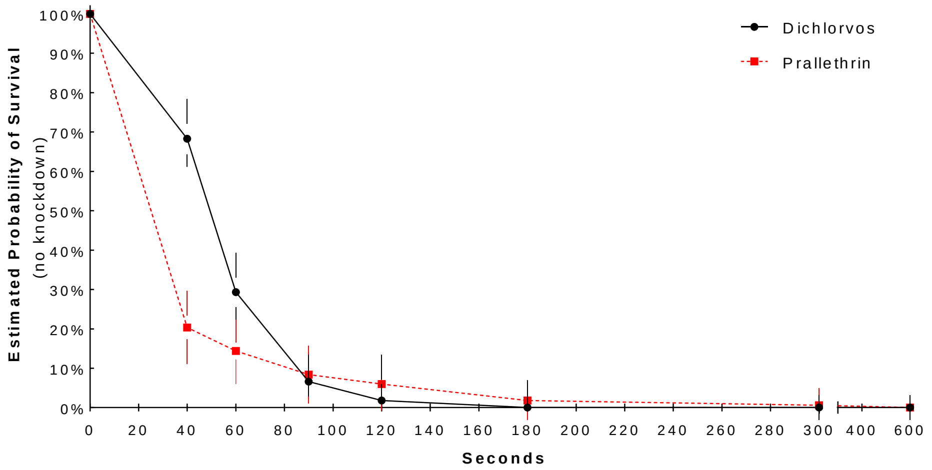


***Figure 1 - Estimated probability of survival (no knockdown) of the AHB under the effect of Dichlorvos (black continuous line) and Prallethrin (red discontinuous line) based on life table methodology. Points represent mean estimated probability of survival (no knockdown) of 167 AHB tested to pralletrin and 167 to dichlorvos (total 334 AHB). Vertical lines represent point wise confidential intervals of 95%.***

*It is possible to verify that at 40 seconds the estimated probability of survival (no knockdown) for prallethrin (red) was approximately 20%, that is, 133 out of a total of 167 of this sample (approximately 80%) were in knockdown in the time interval 0 |- 40 seconds (figure 1 and table 1). On the other hand the estimated probability of survival to dichlorvos (black) was approximately 70%, that is, 53 out of a total of 167 of this sample (approximately 30%) were in knockdown in the time interval 0 |- 40 seconds (figure 1 and table 2). Estimated probability of survival (no knockdown) to prallethrin (red) is lower to dichlorvos (black) up to approximately 90 seconds. After that, the estimated survival for both pesticides became closer and stable. It may also be noted that after 300 seconds almost all AHB were eliminated for both pesticides.*

*Prallethrin showed greater efficiency than dichrovos (p = 0.026). From the analysis of the areas under the curve computed individually for each pesticide was possible to estimate the average survival times of AHB. Under prallethrin was at approximately 58.5 seconds, lower than the average survival estimated for dichlorvos which was estimated at approximately 65,5 seconds.*

*The same can be observed*

*considering the median survival time, calculated by linear interpolation. For prallethrin the median survival was approximately 25.11 seconds whereas for dichlorvos was approximately 49.38 seconds.*

## *DISCUSSION*

*It is important to consider the public policy to avoid accidents by poisonous animals[28] There is a recommendation of the Brazil Minister of Health related to surveillance, prevention and control of zoonoses and accidents caused by poisonous animals[29]. Although some studies have been done on the risk of accidents by AHB in urban area[9-11,30] none of them describes a methodology as well as the effectiveness of pesticides in public health.*

*Recently was published a study[31] about the effect of truck-mounted, ultra low volume (UBV) mosquito adulticides on honey bees (Apis mellifera) in a suburban field setting. This study showed that there is no significant effect of prallethrin and some other pesticides used by UBV to control mosquito on honey bees around the areas that received insecticide. However it showed the indirect effect on AHB resulting to mosquito control. Our study showed the direct effectiveness of prallethrin on AHB by focal spraying when compared to dichlorvos.*

*To effective control AHB is important to use a pesticide with a quick knockdown, so that the bees do not have enough time to defend their hive. Consequently attacks on control team, people and other animals around can be avoided, reducing the risk of accidents.*

*It was not determined the exact knockdown time once during the test healthy AHB tended to aggregate to bees in knockdown state, leading to the use of time intervals in order to reduce errors.*

*The methodology applied in the experiment was validated by the low induction of stress, as the control containers (without spraying pesticide) showed no knockdown by stress. Besides this methodology it was possible to establish a representativeness of the focal spray currently in São Paulo city to control AHB.*

*Dichlorvos can control AHB in time enough to avoid attack of insects and was used as positive control. The alternative pesticide needs to have equal or greater knockdown capability when compared to positive control.*

## *CONCLUSION*

*In this study prallethrin showed more efficiency than dichlorvos. The average and median survival times were lower when compared to dichlorvos. In addition, it is possible to estimate that AHB exposed to prallethrin will be less time to defend their hives (AHB died quicker) besides to present lower toxicity to humans and to the environment.*

*By this study prallethrin showed better effect to control AHB when compared to dichlorvos what is the present pesticide used by Zoonoses of São Paulo city.*

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