Attractiveness and toxicity of two insecticides to *Tetragonula laeviceps* (Apidae: Meliponinae)

N Mubin, I Nurulalia and Dadang
Department of Plant Protection, Faculty of Agriculture, IPB University, Indonesia

Corresponding author email: mubin.nadzirum@apps.ipb.ac.id

Abstract. Stingless bee, *Tetragonula laeviceps*, plays a primary role in ecosystem services as a pollinator for coffee, cocoa, and pepper. Attacks of insect pests cause low production of several plantation plants. Farmers commonly use synthetic insecticides to control insect pests. However, improper use of insecticides often adversely affects the ecosystem and human health, including pollinators. In Indonesia, research on the side effects of insecticides on non-target insects such as flower visitor insects (pollinators) is minimal. This study aimed to evaluate the attractiveness and toxicity of two insecticides to *T. laeviceps*. Stingless bees were obtained from beekeepers at Banten. Y-tube olfactometer test was used to determine the attractiveness of bees to insecticides, and a topical test was used to determine the mortality effect of insecticide on bees. The insecticides used were insecticide A (a.i. alpha-cypermethrin) and insecticide B (a.i. spinetoram) at 100 ppm and 60 ppm, respectively. The results showed that bees were more attracted to insecticide B by 73.3% than insecticide A (26.7%). Insecticide B caused 100% bee mortality after 48 hours after treatment (HAT), while Insecticide A caused 0% bee mortality after 48 HAT. The results indicate that insecticide B attracted stingless bees and has a high mortality level.

Keywords: mortality, olfactometer, stingless bee, topical test

1. Introduction
Almost all tropical rainforest plants are pollinated by animal and one-third of them are pollinated by insects, so when the population of insect pollinator decrease, yields of plants will reduce [1,2]. Bees are social insects that play a primary role in ecosystem services as pollinators. Like *Apis mellifera* and *A. cerana*, Indonesia also has native bees such as the stingless bee *Tetragonula laeviceps* (Apidae: Meliponinae) as potential pollinators in many cultivated plants.

Plantation crops such as coffee, cocoa, and pepper are managed to have self-pollination [3,4]. In many studies, it has been shown that cross-pollination has also occurred among plants where bees were the pollinators and resulted in the high percentage of fruit set and seed set [5,6,7,8], produced a high quality of yield [9], and performed high effectiveness and efficiency [10]. According to Klein et al. [11], cocoa is the most affected by pollinators' loss, which leads to more than 90% yield loss. Moreover, US Environmental Protection Agency (EPA) puts the value of pollinating in the range of US$ 29 billion per year [12].

On the other hand, efforts to increase the production in the plantation are often disrupted by pests attacks. Farmers usually use synthetic insecticides to control the pests so that the plant can produce well. Insecticides with active ingredients such as alpha-cypermethrin and spinetoram are the common insecticide used. Alpha-cypermethrin is classified as group III pyrethroids that serves to disrupt the voltage-gated sodium channel (VGSC) [13,14]. While spinetoram is classified as a spinosyns group that
can cause hyperexcitation from the nervous system [15], so the affected insect pest will have convulsions and intense vibrations and suffocate and die. Inappropriate use of insecticides harms the ecosystem, human health, and pollinators.

Studies showed that the decline of bee populations [16], colony collapse disorder [17,18], and the effect of pesticides [19,20,21], may lead to a loss of diversity, reduced abundance of bees, and interference with the pollinator and plant interaction. However, studies on the hazardous effect of pesticides, such as their attractiveness and toxicity to bees, are still limited in Indonesia. Olfactometry is one of the assessment methods that can be used to evaluate the orientation responses to odor [22,23]. The attraction of bees to odor is the initial step in finding food sources (nectar and pollen). This method is used to determine the attractiveness of bees to odor sources emitted by food sources or insecticides commonly used by the farmer to control insect pests. Toxicity studies are mostly on insect targets but rarely on non-target insects such as bees. So, research on the side effects of insecticides on non-target insects such as flower-visiting insects (pollinators) is needed. This study aimed to evaluate the attractiveness and toxicity of two insecticides to stingless bee T. laeviceps.

2. Materials and Methods

This research was conducted at the Insect Physiology and Toxicology Laboratory, Department of Plant Protection, IPB University, from May to June 2021. Stingless bees, T. laeviceps (Apidae: Meliponinae), were obtained from a beekeeper at Banten.

2.1. Preparation

The bees used as insect tests were caught from the entry hole hive using a glass tube. Then the bees were acclimatized in box gauze 30 cm x 30 cm x 30 cm. Two types of insecticides used were insecticide A and insecticide B containing alpha-cypermethrin and spinetoram as the active ingredients (a.i), respectively.

2.2. Olfactory orientation assay of T. laeviceps

Methods of the response olfactory test of T. laeviceps to two insecticides using olfactometer was undertaken referred to Lu et al. [24] and Li et al. [22]. The odor sources were insecticide A and B with recommended concentrations of 100 ppm and 60 ppm, respectively. One milliliter of insecticide A formulation or 0.5 ml of insecticide B formulation was pipetted and then diluted with water to produce 1 l of insecticide solution. Each insecticide solution was pipetted and then dropped as much as 200 ml on the foam measuring 2 cm x 2 cm x 2 cm then placed in a small tube. The liquid of each insecticide in this test should not be used after more than 20 minutes to avoid volatility. Insecticide-containing foam was put into a glass tube that was connected to the tip of the arm of the Y-tube olfactometer. The glass tubes were covered with gauze then a branch of the Y-tube olfactometer was connected with a hose to the olfactometer pump (Dyna-Pump Model 3). Between of olfactometer and pump were installed flowmeter (Gilmont GF 6541-1215) to check the airflow rate. The bee was placed at the end of the olfactometer arm, and the flowmeter was running with a flow rate of 40 ml/minutes. The test was carried out at room temperature (± 26 °C) and RH 76% (Figure 1)

This test consisted of 10 treatments, i.e. 1) insecticide A vs. honey, 2) insecticide B vs. honey, 3) insecticide A + honey vs. honey, 4) insecticide B + honey vs. honey, 5) insecticide A vs. aquades, 6) insecticide B vs. aquades, 7) insecticide A + honey vs. aquades, 8) insecticide A + honey vs. aquades, 9) insecticide A + honey vs. insecticide B + honey, and 10) insecticide A vs. insecticide B. Insecticide, honey, or aquades were placed on the glass tube B1 or B2 as odor source according to the treatment (see Figure 1). The bees used were acclimatized for 2 hours without feed. Each bee was used only once in the experiment. A total of 15 bees were used for individual test samples.
2.3. Contact toxicity assay of *T. laeviceps*

The contact toxicity was evaluated to the stingless bee *T. laeviceps* by topical application method [25]. The concentrations of insecticide A or B used on the topical test were the same as the olfactory test. A 1 µl - microsyringe was used to drop the insecticide solution [26]. The test consisted of 3 treatments 1) insecticide A 100 ppm, 2) insecticide B 60 ppm, and 3) control (aquades only). To drip the liquid easier, the bees were anesthetized by placing them in the fridge (-10 °C) for 2 minutes [27]. Then, 1 µl of the solution was dripped on the thorax of the bee. Each treatment contained 10 bees and was repeated 3 times. After treatment, the bees were kept in plastic tubes with a diameter of 8.5 cm and a height of 10 cm, and a cotton ball containing 10% honey was placed in the tubes (Figure 2). The observation was carried out 48 hours after treatment (HAT) [46].

![Figure 2](image2.png)

**Figure 2.** Plastic tube for keeping the bees after treatment.

h: height (10 cm), d: diameter (8.5 cm), g: gauze, and rb: rubber band

2.4. Data analysis

In this study, a completely randomized design (CRD) was used. A chi-square test at a 5% level was used to analyze the significant differences in the selection of odor sources in the olfactory test. Data analysis used Minitab 17 Software and Ms. Excel 2016.
3. Results and discussions

Stingless bee, *T. laeviceps* has around 3.44-3.76 mm in size and has a shiny-black body [28] whereas to honey bees (*A. mellifera, A. cerana,* and *A. dorsata*) has a bigger size (10-35 mm) [29]. Different from honey bees who have a stinger, native bees or stingless bees do not have a stinger. However, they have strong mandible that can protect their colony from intruders such as ants, geckos, and flies. Similar to the honey bee, *T. laeviceps* also plays an important role as a pollinator. These small bees help in pollination and increase fruit set and seed set [6,7]. In the field, they are often seen visiting flowers in plantation crops such as coffee (Figure 3b). Most people might misrecognize *T. laeviceps* because its body size and morphology are similar to fly (Figure 3a). Due to its small body, *T. laeviceps* is hardly recognized by farmers. Thus, its role is not widely known.

![Figure 3. Stingless bee T. laeviceps (a) and the bees (yellow arrow) pollinated coffee flower (b).](image)

3.1. Attractiveness and toxicity of *T. laeviceps* to insecticide

The bee *T. laeviceps* is also known as a propolis bee because the bees need the resin to build their hives [30,31,32]. Stingless bees need a food source such as nectar and pollen from blooming flowers to fulfill nutrients in their colony, which is specially managed by worker bees. The resource for bees’ nutrients from plants could be obtained from either wild or intensively cultivated crops. However, the intensively cultivated plants are connected to the applications of synthetic insecticides in controlling insect pests. Based on mentioned fact, practices of insecticides application would affect the population dynamic of *T. laeviceps*.

Figure 4 (a and b) showed that the stingless bees prefer honey and aquades odor more (70%) than insecticides (<30%). Bees were also preferring a single treatment of honey or aquades compared to the insecticides-honey mixture (A or B). Aroma 1 (Insecticide A, Insecticide B, Insecticide A+honey, and Insecticide B+honey) vs aroma 2 (honey) had an average preference of 28.33% vs 71.67%, respectively (Figure 4a). Figure 4b, aroma 1 (Insecticide A, Insecticide B, Insecticide A+honey, and Insecticide B+honey) vs aroma 2 (aquades) had an average preference of 30.0% vs 70.0%, respectively. Honey or water is a source that is needed by bees for life. Bees need honey as food for their offspring, nestmates, drone, and queen, while water is used for mineral extraction or to regulate their nest when conditions are hot outside the hive. The bees will drop the water and move the wings to stabilize the temperature in the nest [33].

Energy from honey is needed by bees. Bees have to visit a minimum of 100 flowers to obtain 17 mg raw honey [34,35]. To produce 1 kg of honey, the bees have to visit about 5.7 million flowers. Bees that visit a lot of flowers that have been sprayed with insecticide have a higher risk of hazard exposure. Bees who visited many flowers sprayed with insecticide have a higher risk of exposure. In contrast, Kessler [36] mentioned that *A. mellifera* and *Bombus terrestris* did not avoid foods containing neonicotinoid (imidacloprid, thiamethoxam, and clothianidin) and the bees also consumed more the sucrose solutions containing imidacloprid and thiamethoxam than without insecticide.

In Figure 4c, it can be seen that insecticide A or B mixed with honey showed a significant difference from the result of insecticide alone. The attractiveness of insecticide A+honey vs. insecticide B+honey to stingless bees was 40 and 60%, respectively. Meanwhile, the attractiveness of insecticide A vs. insecticide B was 26.67 and 73.33%, respectively. The results showed that there was an effect of honey
compared to that without the honey or solely insecticide. Bees have no choice but to visit the flower to collect honey even though the flower has been sprayed with insecticides previously. The food source from sprayed flowers must be obtained to fulfill the needs of the colony. However, strong insecticidal scents were not selected when the plant was not flowering (containing honey). In the tests of insecticide A vs. honey and insecticide B vs. honey (Figure 4a), bees preferred honey to insecticide. On the other hand, in Figure 4c, bees were more attracted to insecticide B (with or without honey) than to insecticide A. EPA [37] stated spinetoram (a.i of insecticide B) as a green label insecticide which means it is safe for non-target insects.

Figure 5 showed that control and insecticide A treatments did not show mortality to the bees at 24 HAT and 48 HAT. In contrast, insecticide B caused 80% and 100% mortality at 24 and 48 HAT, respectively. The result of topical and olfactometer demonstrated a significant relationship between insecticide A vs. insecticide B (Figure 4c). The result of the olfactory test showed that the attractiveness of bees to insecticide B was higher (73.33%) than insecticide A (26.67%). However, the topical test on insecticide B showed the highest mortality effect (100%). EPA states that spinetoram is safe for non-target insects.

**Figure 4.** Response of the *T. laeviceps* test in Y-tube olfactometer in dual choice aroma. N = 15 for each treatment. The percentages of interest were: A: insecticide vs. honey, B: insecticide vs. aquades, and C: Insecticide A vs. Insecticide B. Inst. A (Insecticide A), Inst. B (insecticide B), Inst. A+Honey (insecticide A: Honey 1:1), Inst. B+Honey (insecticide B: Honey 1:1). ** p<0.01, * p<0.05.
Several reports stated that spinetoram and spinosyn have a high specific target, especially to Lepidopteran insects, while non-target insects such as Diptera, Coleoptera, and Hymenoptera have safe levels even to pollinators [38,39]. However, the toxicity of other pesticides such as organophosphates (chlorpyrifos and phosmet) to stingless bee Scautotrigona bipunctata and Tetragonisca fiebrigi (contact and oral test) showed that both pesticides were toxic to bees and especially chlorpyrifos showed a higher toxicity to both bees [40].

In visiting the food source, the smell of flowers is the first factor sensed by the bees. This is related to the finding in the olfactory test in this study that insecticide B alone gave high attractiveness response (Figure 4c). However, when all of the food sources have been contaminated with pesticides, the bees will choose the safe food sources. But, the bees will have difficulties in detecting contact/stomach insecticides without trying it. Therefore, they remained attracted to the insecticides-contaminated food source, and then insecticidal MoA will start taking effect.

Insecticide A is an insecticide with the active ingredient (a.i.) alpha-cypermethrin. Alpha-cypermethrin is a group 3 pyrethroid that inhibits sodium channel modulator that causes sodium channel to open. In some cases, it causes reactions that disrupt the function of the voltage-gated sodium channel (VGSC) [13,14, 41]. In comparison, insecticide B is an insecticide with a.i. spinetoram. Spinetoram is a new spinosyn insecticidal group with MoA 5 that is local or translaminar [15]. It means the spinetoram can penetrate the cuticle of plant tissue where the larvae of Lepidoptera and bugs (Hemiptera) hide. Spinetoram will attack the nervous system and muscles in insects. Allosterically will activate nACHRs (Nicotinic acetylcholine receptor), causing hyperexcitation of the nervous system. Insect pests that are exposed to these pesticides will experience severe convulsions, shake, then limp and die.

Some insecticides are acute and chronic poisons. When bees are exposed to acute insecticide through contact or oral/ingested, the bee will die immediately. Whereas bees exposed to chronic insecticide do not die immediately, they will carry the contaminants to the hive. Bees have trophallactic behavior, i.e. exchange between a worker with a worker, a worker with a drone, or a worker with a queen in a liquid suspension [33]. If bees carry nectar or pollen contaminated with insecticide, then the entire colony will be exposed to those insecticides. According to Mullin et al. [42], as many as 98 pesticides and their metabolites were detected in bee pollen around 214 ppm. Exposure to acute neurotoxic insecticides can reduce the bee’s health. Pistoru et al. [43] stated that as many as 11,500 honey bee colonies from 700 beekeepers experienced symptoms of insecticide poisoning. This is due to the application of clothianidin through sowing and dusting, so the nectar and pollen were contaminated with the insecticide.

Pesticides can cause disorientation on worker bees in returning to their hive (CCD). One of the insecticide active ingredients that caused CCD is imidacloprid (Neonicotinoid group) [44]. This is one
of the causes of the loss of thousands of colonies in Europe and the US (CCD/colony collapse disorder). Efforts are made to make an understanding. The EPA has created a memorandum (MoU) with the pollinator partnership. The goals are to improve the health of pollinator and their habitats [45]. EPA also issues the rule on pesticide risk assessment as a basis for decision-making for all chemical stakeholders. Meanwhile, there is no regulation in Indonesia, but contamination and residue of pesticides can be reduced by communicating with stakeholders, developing best management practices (BMP), and providing alternative forage.

4. Conclusion
Based on the results of olfactory responses, *Tetragonula laeviceps* was more attracted to insecticide B (a.i spinetoram) by 73.3% of attractiveness than insecticide A, a.i alpha-cypermethrin (26.7%). Whereas in topical responses, insecticide B caused 100% bee mortality 48 hours after treatment. So, both the test results showed that insecticide B can attract stingless bee more but has a high mortality level.

Acknowledgment
The authors would like to thank Mr. Nana, who helped in supplying the colony of stingless bee (*T. laeviceps*) from Banten, to Ms. Khalisa Sasti Andina who assisted in this research, to Mr. Prayogo P Asmoro who assisted in data analysis, to Mr. Suryadi and Mr. Marich for helping in making schematic of the Y-olfactometer and taking pictures of the stingless bee.

References
[1] Bawa K S 1990. Plant–pollinator interactions in tropical rain forests. A. Rev. Ecol. Syst 21 399–422.
[2] Kevan P G, Philips T P. 2001. The economic impacts of pollinator declines an approach to assessing the consequences. *Synthesis*. 5(1): 8
[3] Glendinning D R. Natural pollination of cocoa. *New Phytol.* 71: 719-729.
[4] Semple K S. 1974. Pollination in Piperaceae. *Annals of the Missouri Botanical Gardens*. doi.org/10.2307/2395033
[5] Ibrahim G A. 1988. Effects of insect pollinators on fruit set of cocoa flowers. *Proceedings of the International Cocoa Research Conference* 1987, Santo Domingo: 303-306.
[6] Klein A M, Steffan-Dewenter I, Tscharnke T. 2003a. Fruit set of highland coffee increase with the diversity of pollinating bees. *Proc Royal Society*. 270: 955-961. doi 10.1098/rspb.2002.2306
[7] Klein A M, Steffan-Dewenter I, Tscharnke T. 2003b. Bee pollination and fruit set of *Coffea arabica* and *C. canephora* (Rubiaceae). *American J Botani*. 90(1): 153-157. doi 10.3732/ajb.90.1.153
[8] Frimpong E A, Gemmil-Herren B, Gordon I, Kwapong P K. 2011. Dynamic of insect pollinators as influenced by cocoa production systems in Ghana. *Journal of Pollination Ecology*. 5(10):74-80. doi 10.26786/1920-7603/2011(12)
[9] Prado S G, Collazo J A, Irwin R E. 2018. Resurgence of specialized shade coffee cultivation: Effects on pollination services and quality of coffee production. *Agriculture, Ecosystems & Environment*. 265: 567-575. doi.org/10.1016/j.agee.2018.07.002
[10] Layek U, Kundu A, Bisui S, Karmakar P. 2021. Impact of managed stingless bee and western honey bee colonies on native pollinators and yield of watermelon: A comparative study. *Annals of Agricultural Sciences*. 66(1): 38-45.
[11] Klein A M, Vaissie`re B E, James H, Cane J H, Steffan-Dewenter I, Cunningham S A, Kremen C, Tscharntke T. 2007. Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences*. 274: 303-313.
[12] [EPA] US Environmental Protection Agency. 2018. Understanding how pesticide exposure affects honey bee colonies. 30 July 2018.
[13] Tan J, Liu Z, Wang R, Huang Z Y, Chen A C, Gurevitz M, Dong K. 2005. Identification of amino acid residues in the insect sodium channel critical for pyrethroid binding. *Mol. Pharmacol.* 67:513–522.

[14] Singh A K, Tiwari M N, Prakash O, Singh M P. 2012. A current review of cypermethrin-induced neurotoxicity and nigrostriatal dopaminergic neurodegeneration. *Current Neuropharmacology.* 10(1):64-71. doi: 10.2174/157015912799362779

[15] [IRAC] Insecticide Resistance Action Committee. 2020. IRAC Mode of Action Classification Scheme: Spinetoram. Crop Life International. p6.

[16] National Research Council. 2007. *Status of pollinators in North America.* Washington, DC: The National Academies Press.

[17] Olroyd B P. 2007. What's killing American honey bees?. *PloS Biol.* 5(6):e168. Doi: 10.1371/journal.pbio.0050168

[18] Vanengelsdorp D, Evans J D, Saegerman C, Mullin C, Haubruege E, Nguyen B K, Frazier M, Frazier J, Cox-Foster D, Chen Y, Underwood R, Tarpy D R, Pettis J S. 2009. Colony collapse disorder: a descriptive study. *Plos One.* 4(8):e6481. doi: 10.1371/journal.pone.0006481

[19] Sanchez-Bayo F, Goka K. 2014. Pesticide residues and bees – a risk assessment. *Plos One.* https://doi.org/10.1371/journal.pone.0094482

[20] Park M G, Blitzer E J, Gibbs J, Losely J E, Danforth B N. 2015. Negative effects of pesticides on wild bee communities can be buffered by landscape context. *Proc Biol Sci.* 282(1809). Doi: 10.1098/rspb.2015.0299

[21] Kandida K. 2017. Analisis tingkat pencemaran pestisida pada madu lebah (*Apis cerana*) tanaman kopi. Bengkulu: Bengkulu University.

[22] Li Y, Zhong S, Qin Y, Zhang S, Gao Z, Dang Z, Pan W. 2014. Identification of plant chemicals attracting and repelling whiteflies. *Arthropod-Plant Interactions.* 8: 183–190

[23] Asmoro PP, Dadang, Pudjianto, Winasa IW. 2020. Olfactory RESPONSE of *Plutella xylostella* (Lepidoptera: Yponomeutidae) adults to refugia plant. *ICoSA, IOP Conf. Series: Earth and Environmental Science.* IOP Publishing. doi:10.1088/1755-1315/752/1/012039

[24] Lu J, Liu S, Shelton A M 2004 *Bull Entomol Res* 94:509-596 DOI: 10.1079/BER2004

[25] Park C G, Shin E, Kim J. 2016. Insecticidal activities of essential oils, Gaultheria fragrantissima and Illicium verum, their components and analogs against *Plutella xylostella* (Lepidoptera: Yponomeutidae) adults to refugia plant. *ICoSA, IOP Conf. Series: Earth and Environmental Science.* IOP Publishing. doi:10.1088/1755-1315/752/1/012039

[26] [EPA] Environmental Protection Agency. 2014. *Guidance for Assessing Pesticide Risks to Bees.* Washington DC: The United States Environmental Protection Agency.

[27] Kinash I, Nugraha R S, Putra R E, Permana A D, Rosimati M. 2017. Toksisitas beberapa jenis fungisida komersial pada serangga penyerbuk, *Trigona (Tetragonula) laeviceps* Smith. *J Entomologi Indonesia.* 14(1): 29-36. doi 10.5994/jei.14.1.29

[28] Trianto M, Marisa F, Kisman M D. 2020. *Tetragonula laeviceps* (Hymenoptera: Apidae: Meliponini): Morphology, Morphometric, and Nest Structure. *Bioeduscience.* 4(2): 188-194. doi: 10.22236/ji.2024.2

[29] Niem NV, Trung Q. 1999. Morphological comparison of three Asian native honey bees (*apis cerana, a. dorsata, a. florea*) in northern Vietnam and Thailand. *Biotropia.* 14: 10-16.

[30] Roubik D W. (1989) Ecology and natural history of tropical bees, Cambridge University Press, New York

[31] Leonhardt S D, Blüthgen N. 2009. A sticky affair: resin collection by Bornean Stingless Bees. *Biotropica.* 41(6), 730–736. doi:10.1111/j.1744-7249.2009.00535.x

[32] Gruter C. 2020. *Stingless Bee.* Springer. 87-130p.

[33] Wilson E O. 1971. *The Insect Societies.* Belknap Press.

[34] Ribbands C R. 1949. The foraging method of individual honey bees. *J Anim Ecol.* 18:47-66.

[35] Lundie A E. 1925 *The flight activities of honey bee.* USDA Bull
[36] Kessler S C, Tiedeken E J, Simcock K L, Derveau S, Mitchell J, Softley S, Stout J C, Wright G A. 2015. Bees prefer foods containing neonicotinoid pesticides. Nature 521(7550), 74–76

[37] [EPA] US Environmental Protection Agency. 2008. Green chemistry challenge award recipients by technology. https://www.epa.gov/greenchemistry/green-chemistry-challenge-award-recipients-technology

[38] Miles M, Mayes M, Dutton R. 2002. The effects of spinosad, a naturally derived insect control agent, to the honeybee (Apis melifera). Meded Rijksuniv Gent Fak Landbouwkd Toegep Biol Wet. 67(3):611-6.

[39] Besard L, Mommaerts V, Abdu-Alla G, Smagghe G. 2011. Lethal and sublethal side-effect assessment supports a more benign profile of spinetoram compared with spinosad in the bumblebee Bombus terrestris. Pest Manag Sci. 67(5):541-7. doi: 10.1002/ps.2093.

[40] Dorneles A L, Rosa A S, Blochtein B. 2017. Toxicity of organophosphorus pesticides to the stingless bees Scaptotrigona bipunctata and Tetragonisca fiebrigii. Apidologie, 48 (5): 612-620. doi: 10.1007/s13592-017-0502-x

[41] [IRAC] Insecticide Resistance Action Committee. 2020. IRAC mode of action classification scheme: Alpha-cypermethrin. Crop Life International. p6.

[42] Mullin C A, Frazier M, Frazier J L, Ashcraft S, Simond R, Engelsdorp D, Pettis J S. 2010. High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. PloS ONE. 5(3):1-19.

[43] Pistoru J, Bischoff G, Heimbach U, Stahler M. 2009. Bee poisoning incidents in Germany in spring 2008 caused by abrasion of active substance from treated seeds during sowing of maize. Hazards of pesticides to bees – 10th International Symposium of the ICP-Bee Protection Group. 118-127.

[44] van der Sluijs J P, Simon-Delso N, Goulson D, Maxim L, Bonmatin J, Belzunces L P. 2013. Neonicotinoids, bee disorders and the sustainability of pollinator services. Current Opinion in Environmental Sustainability. 5(3):293-305.

[45] [EPA] US Environmental Protection Agency. 2021. Memorandum of understanding between EPA and pollinator partnership. 5pp.

[46] OECD. 1998. Guidelines for the testing of chemicals. Number 213. Honeybees, acute oral toxicity test, in OECD Environmental Health Safety Division (Ed.), Paris