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(Original Article)

Evaluating the Impact of Neonicotinoid and Sulfoximine Pesticides on Honey Bee Workers, *Apis mellifera* L., under Laboratory Conditions

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Abstract

Application of pesticides can have a negative impact on pollinating honey bees, *Apis mellifera* L., ranging from sublethal to lethal concentrations. Consequently, it is important to comprehend any possible impacts of pesticides. Herein, we evaluated the toxicity of specific pesticides (Sulfoxaflor, thiamethoxam, imidacloprid, and acetamiprid) on *A. mellifera* workers after exposure of 24, 48, 72, and 96 hrs under laboratory conditions. As a result, sulfoxaflor was considered the most toxic compound among the tested pesticides after 24-h of exposure. Further, acetamiprid was found least toxic pesticide. The same trend of toxicity on *A. mellifera* was observed after 48, 72, and 96 hrs of exposure. These results of the current investigation suggested that sulfoxaflor might be harmful to honey bee workers.

This demonstrates the extraordinary sensitivity of local honey bee to routinely applied agricultural pesticides, which may have an impact on the colony level due to the extensive usage of these pesticides in Egypt.

Keywords: *Apis mellifera; Sulfoxaflor; Neonicotinoid pesticides; Pollinators; Toxicity*

Introduction

Honey bees, *Apis mellifera* L., are one of the most prevalent and vital animal pollinators (Barascou et al., 2023; Peng *et al*., 2023). Importantly, *A. mellifera* pollinate more than 85% of all cross-pollinated plant species worldwide, accounting for more than 90% of the total pollination (Hung *et al*., 2018). The ability to hover over and pollinate such a wide variety of floral plant species comes with a curse for these bees because they must constantly deal with environmental stresses like parasites, predators, diseases, chemicals, and pesticides (Kumar *et al*., 2020). Furthermore, *A. mellifera* productions are essential to human health. In many countries, particularly Egypt, colony populations have declined dramatically over the previous decade (Al Naggar *et al*., 2018). The most important pollinators for honey bees are agricultural crops (UNEP, 2010). Despite this, however, there is a close relationship between the social and environmental causes that led to the disturbance of the honey bee colony globally (UNEP, 2011). Different agro-chemicals like, pesticides, have caused a devastating effect on honey bees and their

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colonies at a global level (Ostiguy *et al*., 2019). Because bees feed in a variety of flowering plants with different physiologies when they travel throughout this huge area in search of pollen and nectar to satisfy the colony's requirements for carbohydrates, However, these food sources are not always entirely safe; occasionally, they may contain a combination of frequently used, dangerous agro-compounds or chemicals derived from other plants (Requier *et al*., 2020). However, because so many chemicals are being overused, incidences of *A. mellifera* poisoning have increased in frequency in recent years. *A. mellifera* populations have significantly decreased as a result of overuse of pesticides in several nations throughout North America, Europe, and Asia (Gross, 2011; Lundin *et al*., 2015). The poisoning of bee pollinators is another significant negative environmental impact of chemicals that ultimately causes farmers and beekeepers to lose a significant amount of cash. 80% of the 264 cultivable plant species depend on bee pollination, hence this incidence disturbs the farming industry tremendously (European Food Safety Authority, 2012). Due to insufficient pollination, these colony losses could be expensive for the beekeeping sector and negatively impact agriculture production and quality (Stein *et al*., 2017). *A. mellifera* are exposed to a wide range of xenobiotics, both from natural and artificial sources. However, the polluted floral nectar can be brought back to the colony by bee foragers to be used as food or stored as a resource for future generations. Agricultural pesticides kill bees in a variety of ways, including by killing foraging workers directly with their acute toxicity, making the entire colony more vulnerable to pathogens, and eventually reducing their ability to grow in the natural environment by building up in the pollen inside the colony. Nectar from several flowering plant species contains chemicals generated by plants that are toxic to various pollinators (Zhu *et al*., 2015). One of the most often utilized classes of pesticides is neonicotinoids. Around 20,000 tons of active ingredients were employed globally in 2010, accounting for around one-third of all pesticide treatments (Bonmatin *et al*., 2015). Neonicotinoid pesticides are effective at controlling pests in a variety of agricultural crops, but they can also kill non-target species such as pollinators in addition to pest insects (Aguiar *et al*., 2023). *A. mellifera* sublethal effects have been a particular source of concern because they are neurotoxicants. This pesticide family marked a watershed moment for integrated pest- and resistance-control programmes. Imidacloprid, acetamiprid, sulfoxaflor, clothianidin, thiamethoxam, thiacloprid, dinotefuran, nithiazine, and nitenpyram are examples of neonicotinoid pesticides (Fairbrother *et al*., 2014). The neonicotinoids are blamed for the decline in the bee population. They are agonists of nicotinic acetylcholine receptors, which lead to disruption of acetylcholine receptor signaling and neurotoxicity (Odemer *et al*., 2023). Numerous behavioral investigations linked neonicotinoids exposure to detrimental effects on reproduction and foraging behaviors of *A. mellifera* (Verena *et al*., 2016).

In the present study, we assessed the toxicity of certain pesticides (sulfoxaflor, thiamethoxam, imidacloprid, and acetamiprid) at different concentrations on *A. mellifera* workers after 24, 48, 72, and 96 hrs of exposure under laboratory conditions.

Materials and Methods

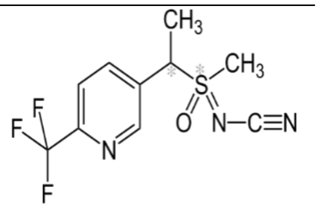
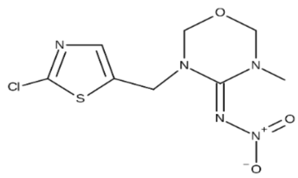
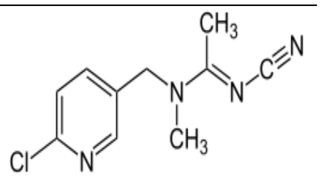
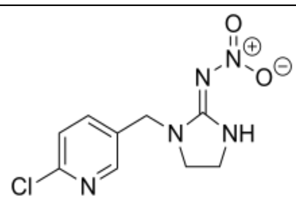
Experimental location

The experiments were carried out at Plant Protection Department, Faculty of Agriculture, Assiut University, Assiut, Egypt.

Honey bee collection techniques

During the active season of August 2022, *A. mellifera* were collected between the hours of 9:00 AM and 12:00 PM from the beehives of the farm apiary of the Faculty of Agriculture, Assiut University. Into clear plastic cups, the bees are shaken from the frames (Evans *et al*., 2009). Each cup featured a hole in the bottom for *A. mellifera* to feed on sucrose solution, and a wire filter was put over the plastic cup spouts for ventilation. To simulate the environment of *A. mellifera* colony in the natural, wax comb was put in containers. It was brought to the lab and incubated there at 34.5 °C and 65% RH (Williams *et al*., 2013).

Table 1. Selected pesticides used in the laboratory experiments

Trade name	Common name	Classification	Chemical	
			Name	Structure
Closer 24% SC	Sulfoxaflor	Sulfoximine	[methyl-oxo-[1-[6-(trifluoromethyl)pyridin-3-yl]ethyl]-λ^6-sulfanylidene]cyanamide	
Actara 24% WG	Thiamethoxam	Neonicotinoid	{3-[(2-Chloro-1,3-thiazol-5-yl)methyl]-5-methyl-1,3,5-oxadiazinan-4-ylidene}nitramide	
Kazaplan 20% SP	Acetamiprid	Neonicotinoid	N-[(6-chloro-3-pyridyl)methyl]-N'-cyano-N-methyl-acetamidine	
IMI Power 35% SC	Imidacloprid	Neonicotinoid	N-{1-[(6-Chloro-3-pyridyl)methyl]-4,5-dihydroimidazol-2-yl}nitramide	

Pesticides

To combat various pests in the Egyptian agro-ecosystem, pesticides were chosen as the most popular application method by farmers. Four of them were selected in this study: sulfoxaflor (closer 24% SC) was purchased from Perfect Co., imidacloprid (IMI POWER 35% SC) was purchased from Kanza Group, thiamethoxam (Actara 25% WG) was purchased from Agrochem Co., acetamiprid

(Kazaplan 20% SP) was purchased from Macca for Agricultural Development Co. (Table 1).

Toxicity bioassay

In order to evaluate for potential detrimental effects on hypopharyngeal survival, we used an exposure scenario for 4 days with lethal and sublethal amounts of pesticides to mimic natural pesticide exposure. To produce the proper concentrations (Table 2), four concentrations of specially prepared pesticides were diluted. Ten bees were housed in each of the three cages (three replicates) that made up each treatment. A syringe (5 cm3) was used to inject the resulting dilution into the upper side of the bee cages, and it was changed every 24±2 hours. The mortality percent of *A. mellifera* workers was recorded after 24, 48, 72, and 96 hrs of treatment. Sugar syrup (one sugar to two cups of water) was the only thing utilized as a control. Each bioassay was repeated twice. The LC_{50}, LC_{90}, and slope values were calculated using Probit regression analysis software by using IBM SPSS Statistics V25 software (SPSS Inc., Chicago, IL) and expressed in µg/ml. The toxicity index was determined using Sun (1950) equations:

Toxicity index = [(LC value of the most toxic tested pesticide / LC value of the tested pesticide) × 100].

Table 2. Selected pesticides and their concentrations

Pesticides	Concentrations (µg/ml)
Sulfoxaflor	0.058
	0.029
	0.014
	0.007
Thiamethoxam	0.244
	0.122
	0.061
	0.03
Acetamiprid	300
	150
	75
	37.5
Imidacloprid	65.62
	32.81
	16.3
	8.2

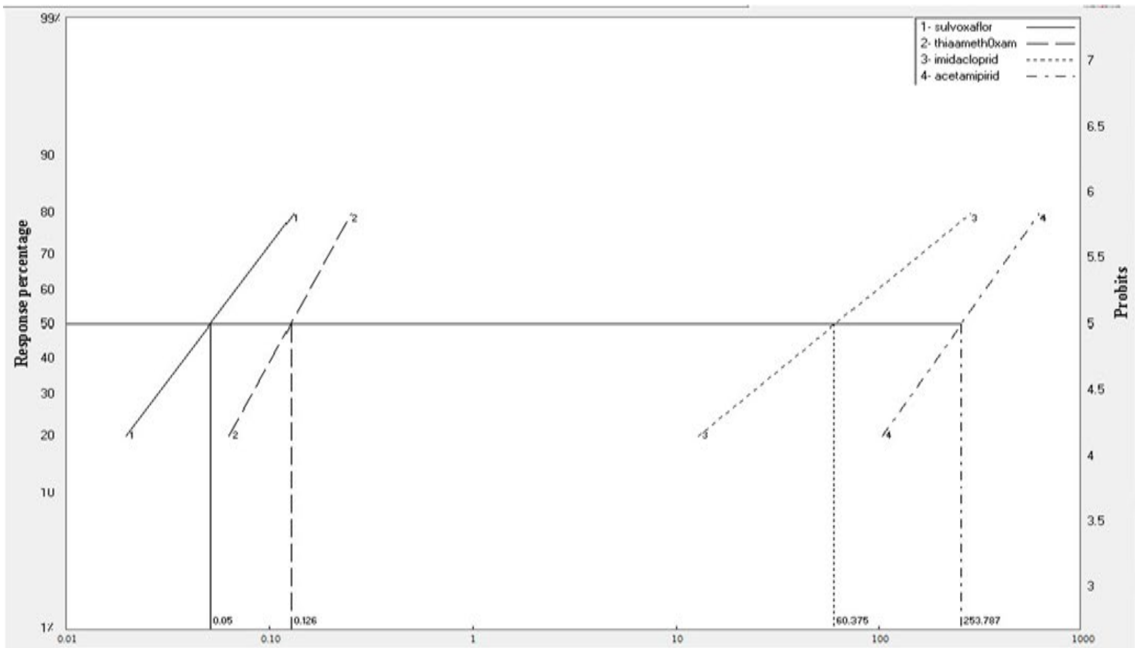
Results and Discussions

The toxicity of the tested pesticides (Table 3 and Fig. 1) stated that among all tested pesticides sulfoxaflor was recorded, the most toxic compound with LC_{50} value of 0.05 µg/ml and LC_{90} = 0.21 µg/ml and the toxicity index for both LC_{50} and LC_{90} was 100. On the other hand, acetamiprid showed the least toxicity level with LC_{50} = 253.78 µg/ml and, LC_{90} = 808.91 µg/ml with toxicity index; 0.02 and 0.02, respectively.

Table 3. Probit analysis parameters of selected pesticides on *A. mellifera* after 24-h of exposure

Pesticides	n[a]	LC_{50} (FL)[1]95%	Toxicity index (LC_{50})[2]	LC_{90} (FL)[1]95%	Toxicity index (LC_{90})[2]	Slope (± SE)
Sulfoxaflor	150	0.05 (0.042-0.066)a	100	0.21 (0.141-0.410)a	100	2.05 (± 0.24)
Thiamethoxam	150	0.12 (0.086-0.213)b	40.48	0.36 (0.213-1.652)ab	59.16	2.80 (± 0.25)
Imidacloprid	150	60.37 (44.719-99.437)c	0.08	130 (105.402-178.426)c	0.16	0.18 (± 0.003)
Acetamiprid	150	253.78 (172.128-20980.796)d	0.02	808.91 (372.554-33254.081)d	0.02	2.63 (± 0. 31)

1FL: fiducial limits, 2 toxicity index = [(LC_{50} or LC_{90} of the most efficient tested pesticide/LC_{50} or LC_{90} of the tested pesticide) x 100]. LC_{50} and LC_{90} values having different letters are significantly different (95% FL did not overlap)



Concentrations (µg/ml)

Fig. 1. LCP lines of selected pesticides on *A. mellifera* after 24-h of exposure

Increasing hours of exposure to pesticides more than the first treatment (24-h) stated that, the toxicity of sulfoxaflor (Table 4 and Fig. 2) still the highest one on *A. mellifera* after 48 hrs. and stayed on the same line of first treatment result with an acute toxicity effect with LC_{50} value of 0.02 and LC_{90} = 0.05 µg/ml and with toxicity index; 100 for both LC_{50} and LC_{90}. Regarding the least toxic one, acetamiprid

still in the same acute level of the previous treatment with LC_{50} value of 152.55 µg/ml and LC_{90} = 489.12 µg/ml, with toxicity index; 0.01 and 0.01, respectively.

Table 4. Probit analysis parameters of selected pesticides on *A. mellifera* after 48-h of exposure

Pesticides	n^a	LC_{50} (FL)[1]95%	Toxicity index (LC_{50})[2]	LC_{90} (FL)[1]95%	Toxicity index (LC_{90})[2]	Slope (± SE)
Sulfoxaflor	150	0.02 (0.014-0.032)a	100	0.05 (0.026-0.367)a	100	3.10 (± 0.25)
Thiamethoxam	150	0.07 (0.031-0.176)b	26.92	0.17 (0.100-6.956)ab	29.72	3.49 (± 0.28)
Imidacloprid	150	21.39 (16.703-27.024)c	0.09	123.71 (84.553-227.561)c	0.04	1.50 (± 0.204)
Acetamiprid	150	152.55 (63.984-3000.304)d	0.01	489.12 (216.798-108256.510)cd	0.01	2.53 (± 0.24)

1FL: fiducial limits, 2 toxicity index = [(LC_{50} or LC_{90} of the most efficient tested pesticide/LC_{50} or LC_{90} of the tested pesticide) x 100]. LC_{50} and LC_{90} values having different letters are significantly different (95% FL did not overlap)

Concentrations (μg/ml)

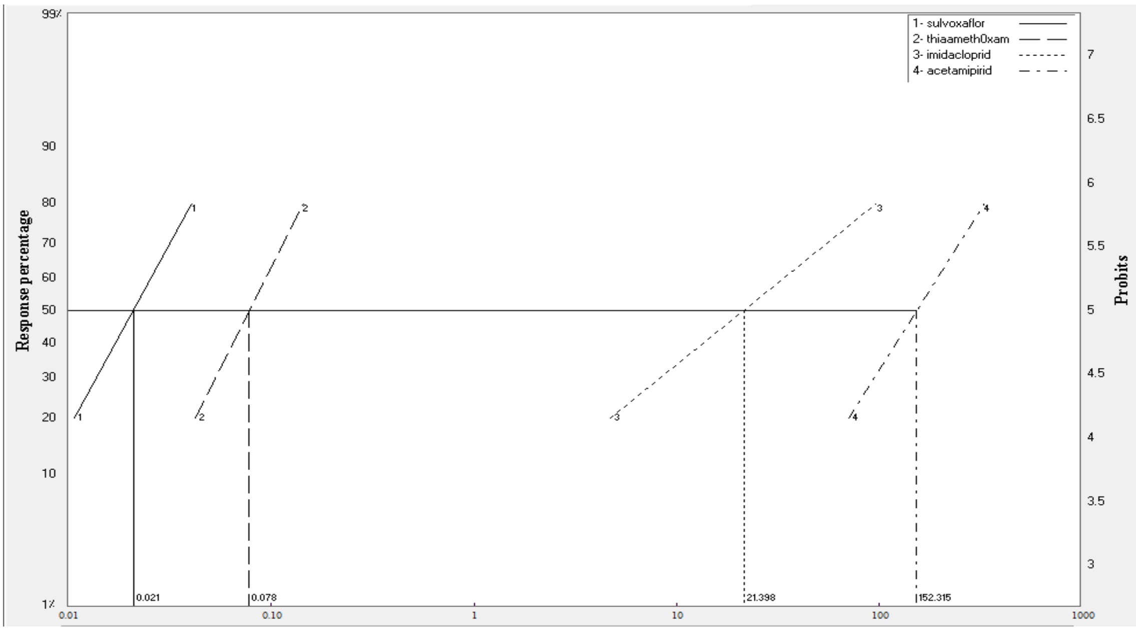


Fig. 2. LCP lines of selected pesticides on *A. mellifera* after 48-h of exposure

After 72-h of exposure to selected pesticides, the toxicity of sulfoxaflor (Table 5 and Fig. 3) still the highest one on honey bee workers and remain in the same direction of first and second treatment result with an acute toxicity effect with LC_{50} value of 0.01 and LC_{90} = 0.04 µg/ml, and with toxicity index; 100 for both LC_{50} and LC_{90}. Regarding the least toxic one, acetamiprid still in the same acute level of the previous treatment with LC_{50} value of 112.32 µg/ml and LC_{90} = 290.89 µg/ml, with toxicity index; 0.01 and 0.01, respectively.

Table 5. Probit analysis parameters of selected pesticides on *A. mellifera* after 72-h of exposure

Pesticides	n^a	LC_{50} (FL)[1]95%	Toxicity index (LC_{50})[2]	LC_{90} (FL)[1]95%	Toxicity index (LC_{90})[2]	Slope (± SE)
Sulfoxaflor	150	0.01 (0.003-0.039)a	100	0.04 (0.022-59.720)a	100	2.84 (± 0.25)
Thiamethoxam	150	0.07 (0.020-0.151)ab	21.13	0.16 (0.090-15.297)ab	26.87	3.25 (± 0.27)
Imidacloprid	150	8.01 (4.031-16.509)c	0.18	34.31 (17.705-138.541)c	0.12	2.12 (± 0.26)
Acetamiprid	150	112.32 (37.601-1325.017)d	0.01	290.89 (138.314-293760.120)cd	0.01	3.00 (± 0. 25)

1FL: fiducial limits, 2 toxicity index = [(LC_{50} or LC_{90} of the most efficient tested pesticide/LC_{50} or LC_{90} of the tested pesticide) x 100]. LC_{50} and LC_{90} values having different letters are significantly different (95% FL did not overlap)

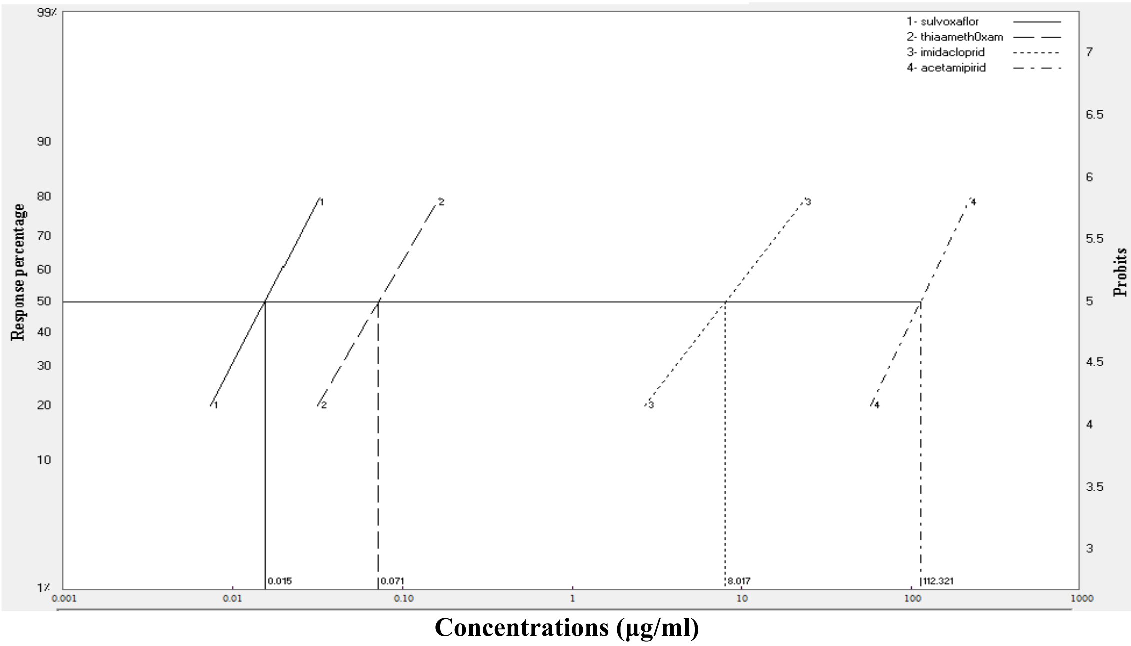


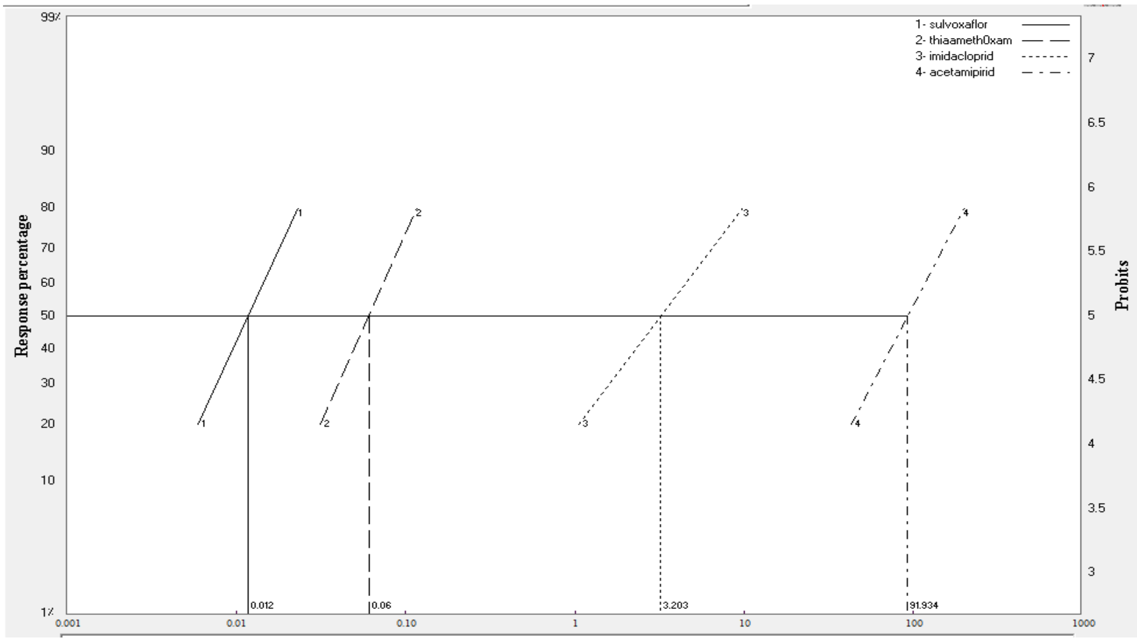
Fig. 3. LCP lines of selected pesticides on *A. mellifera* after 72-h of exposure.

Data in Table 6 and Fig. 4 demonstrated that sulfoxaflor is the highest toxic pesticide among all tested groups during the four experimental exposure periods with LC_{50} value of 0.012 and LC_{90} = 0.028 µg/ml with toxicity index 100 for both LC_{50} and LC_{90}. Certainly, acetamiprid results demonstrated its lowest toxicity on *A. mellifera* workers in all treatments with LC_{50} = 91.93 and LC_{90} = 263.54 µg/ml. Generally, the slope values after 96-h of exposure were ranged between 2.73 and 3.40 for all data revealed that adult of *A. mellifera* workers were relatively homogenous.

Table 6. Probit analysis parameters of selected pesticides on *A. mellifera* after 96-h of exposure

Pesticides	n^a	LC_{50} (FL)[1]95%	Toxicity index (LC_{50})[2]	LC_{90} (FL)[1]95%	Toxicity index (LC_{90})[2]	Slope (± SE)
Sulfoxaflor	150	0.012 (0.011-0.013)a	100	0.028 (0.024-0.034)a	100	3.40 (± 0.31)
Thiamethoxam	150	0.06 (0.011-0.14)ab	20	0.14 (0.08-125.64)b	19.58	3.31 (± 0.28)
Imidacloprid	150	3.21 (1.38-5.43)c	0.37	13.05 (9.57-17.31)bc	0.25	3.17 (± 0.62)
Acetamiprid	150	91.93 (2.97-728.91)cd	0.01	263.54 (119.91-429561.30)d	0.02	2.73 (± 0.24)

[1]FL: fiducial limits, [2] toxicity index = [(LC_{50} or LC_{90} of the most efficient tested pesticide/LC_{50} or LC_{90} of the tested pesticide) x 100]. LC_{50} and LC_{90} values having different letters are significantly different (95% FL did not overlap)



Concentrations (µg/ml)

Fig. 4. LCP lines of selected pesticides on *A. mellifera* after 96-h of exposure.

In the present study, we assessed the toxicity of four pesticides which are highly recommended to apply in the Egyptian agro-ecosystem on *A. mellifera* workers under laboratory conditions. Based on mortality after 24, 48, 72 and 96 hrs of exposure, sulfoxaflor was the most toxic compound followed by, thiamethoxam, imidacloprid and acetamiprid.

This order showed slight changed in the last treatment exposure period of 96 hrs that sulfoxaflor recorded the highest toxicity with LC_{50} value of 0.012 µg/ml and LC_{90} = 0.028 µg/ml followed by thiamethoxam with LC_{50} value 0.06 µg/ml and LC_{90} = 0.143 µg/ml then imidacloprid with LC_{50} value of 3.21 µg/ml and LC_{90} = 13.05 µg/ml then imidacloprid with LC_{50} value of 91.93 µg/ml and LC_{90} = 263.5 µg/ml. Based on how pesticides are classified by the (US-EPA, 2019), bees are considered non-target insects. As a result, all of the tested are regarded as being extremely harmful to *A. mellifera*.

These findings indicated that pesticides that tested were highly toxic to *A. mellifera* workers after 24, 48, 72 and 96 hrs of exposure. The same results were also found by Babcock *et al*., (2011) and Watson *et al*., (2011). Sulfoxaflor's LC_{50} value in *A. mellifera* foragers was found to be 12 ppb in the investigation (Siviter *et al*., 2018). Colony growth and activity were lowered by chronic exposure to a sublethal concentration of sulfoxaflor by reducing pollen deposition as well as honey output. Additionally, *A. mellifera* was particularly sensitive to thiamethoxam (Bruna *et al*., 2020). Significant impacts (decreases in bees, brood) were seen following exposure to the two highest dosage rates, and colony loss occurred at the two highest dose rates. Thiamethoxam 50–100 ng/g sucrose solution (Thompson *et al*., 2019) *A. mellifera* exposed to syrup contaminated with 125 g/L imidacloprid reported substantial mortality rates (up to 45%), had imidacloprid residues in their bodies ranging from 2.7 to 5.7 ng/g, and displayed strange behaviors (restless, lethargic, trembling, and falling over) (Sánchez-Bayo *et al*., 2017). The outcomes from the laboratory experiments demonstrate that acetamiprid is harmful to *A. mellifera*. The signs of neurotoxicity and the first deaths happen 15 minutes after ingesting high quantities and between 30 and 45 minutes after being exposed to pesticides by touch. Mortality rates rise with concentration and exposure time (Mazi *et al*., 2020). Since imidacloprid has more fatal effects than other pesticides, a significant mortality rate was seen at the highest dose (Pervez *et al*., 2021). Imidiacloprid dramatically raised AChE and TP levels, according to biochemical studies (Pervez *et al*., 2021). Imidacloprid is the most poisonous compound studied and has significant harmful effects on all *A. mellifera* research parameters (Pervez *et al*., 2021). Results showed that thiamethoxam was exceedingly hazardous to adult of *A. mellifer*a which is considered an indicator of how the bees were exposed to pesticides. The bees were most harmful when acetamiprid and chloride were sprayed directly on them (Costa *et al*., 2014). Furthermore, another result showed that after 24-h of exposure, the LC_{50} values (mg/L) for each tested pesticide were as follows: thiamethoxam, (0.009 mg/L); imidacloprid, (0.003 mg/L) (Abbassy *et al*., 2020). By feeding method, neonicotinoid pesticides were typically the most hazardous to bees (Abbassy *et al*., 2020). Numerous neonicotinoid pesticides have been linked to poisoning symptoms that resembled those seen in the studies (Decourtye and Devillers, 2010). Even though it becomes somewhat less hazardous at lower doses, thiamethoxam is still extremely poisonous when ingested and when in contact with the skin. Thiamethoxam showed a degree of risk long after administration in the indirect contact test, where it was fatal at a concentration 20 times lower than the field one (Tomlin, 2003).

Conclusion

The results demonstrated that the tested pesticides are toxic to *A. mellifera* and may lead to more serious problems later on the behavior and environment of *A. mellifera*, which affects the quality and efficiency of plant pollination as well as the impact on honey bee products. Since abuse of very dangerous pesticides is prohibited, it is imperative to enforce existing laws, manage and regulate the marketing of illicit pesticides, implement policies to do so, and establish strict standards for the registration and marketing of less harmful goods. The illicit import, sale, and distribution of those dangerous pesticides should thus be strictly regulated by the law or at least adhere to the precautions and warnings for the use of these pesticides.

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تقييم تأثير مبيدات النيونيكوتينويد والسلفوكسامين على شغالات نحل العسل *Apis mellifera* L. تحت الظروف المعملية

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الملخص

يؤثر الاستخدام الغير مسؤول لمبيدات الافات تأثيرا سلبيا على نحل العسل *Apis mellifer*a L. كملقحات، والذي يتراوح من التركيزات التحت مميتة إلى التركيزات المميتة. وبالتالي، من المهم فهم أي آثار ضارة محتملة لمبيدات الآفات على نحل العسل. في هذه الدراسة، تم تقييم سمية مبيدات افات مختارة (سلفوكسافلور، ثياميثوكسام، إيميداكلوبريد، وأسيتاميبريد) على شغالات نحل العسل بعد التعرض لمدة 24، 48، 72، 96 ساعة تحت الظروف المعملية. ونتيجة لذلك، كان مبيد السلفوكسافلور أكثر المركبات سمية من بين مبيدات الآفات المختبرة بعد 24 ساعة من التعرض. علاوة على ذلك، كان مبيد الأسيتاميبريد الأقل سمية. وقد لوحظ نفس الاتجاه فى السمية على شغالات نحل العسل المختبرة بعد 48 و72 و96 ساعة من التعرض للمبيدات. وتشير نتائج الدراسة إلى أن مبيد السلفوكسافلور قد يكون ضارًا لشغالات نحل العسل. كما يوضح هذا الحساسية غير العادية لنحل العسل المحلي تجاه مبيدات الآفات الزراعية التي يتم استخدامها بشكل روتيني، والتي قد يكون لها تأثير على مستوى الخلية بسبب الاستخدام المكثف والعشوائى لهذه المبيدات في مصر.