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(Original Article)

Toxicological Impact of Certain Pesticides on Honeybee, *Apis melliferea* L. (Hymenoptera: Apidae) under laboratory Conditions

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Abstract

Honeybees exposed to pesticides direct or indirect ways through their food research trips. Herein, this study examined the toxicity effects on laboratory of seven tested pesticides insecticides from different pesticide groups. emamectin benzoate, imidacloprid, chlorpyrifos, indoxacarb, lambada cyhalothrine, glyphosate and thiophanate-methyl on honeybee workers, *Apis mellifera* L. through different exposure periods of time at 24, 48, and 72 hours. The selected pesticides were ranked based on their toxicity from the most to least one as following: emamectin benzoate, imidacloprid, chlorpyrifos, indoxacarb, and lambda cyhalothrine. Further, thiophanate-methyl and glyphosate were the lowest toxicity among selected pesticides. The highest toxic pesticide during the exposure period 24, 48 and 72 hrs was emamectin benzoate with LC_{50} values (0.247, 0.047 and 0.020 ppm) and with LC_{90} values (5.752, 0.302 and 0.072 ppm) respectively, and the least one was glyphosate with LC_{50} values of (6861.151, 3366.968 and 2477.267 ppm) and LC_{90} values of (28243.795, 9033.695 and 6203.485 ppm) after 24, 48 and 72hr of exposure respectively. However, chlorpyrifos ranked the third toxic pesticide at 24 and 48 hrs of treatment with LC_{50} values of 10.226 and 2.731 ppm and with LC_{90} values of (101.224 and 7.496 ppm for 24 and 48 hrs, respectively). Whereas, after 72 hrs chlorpyrifos recorded the fourth toxic pesticide with LC_{50}2.086 ppm and LC_{90} 5.179ppm. Based on the toxicity index, results showed that, the most to least toxic pesticides were arranged as followed: emamectin benzoate>imidacloprid >chlorpyrifos >indoxacarb >lambada cyhalothrine> thiophanate -methyl > glyphosate. These results demonstrated that pesticides are very toxic to honeybee workers and must avoid applying them during the times when bees are most active such as during flowering periods.

Keyword: *Honeybee, Pesticide hazards, Toxicity, Pesticides*

Introduction

Honeybee, *Apis melliferea* L. is one of the most significant distributed pollinators worldwide and about 90% of all plant species pollinated by its workers

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(Gbylik-Sikorska *et al*., 2015). It produces several valuable products for human consumption such as pollen, royal jelly, bee venom, beeswax, and honey (Al Naggar *et al*., 2021). Additionally, the previous products are mainly involved in other commercial industries products among are cosmetics and pharmaceutical. The forage honeybee's population affected in two ways for example: the direct exposure of applied insecticides which significantly reduces and kills bee workers, whereas the indirect damage appears as insecticides residues in the products of tolerant honeybee's strains to different insecticides doses.

Recently, honeybee's workers sharply decreased in population numbers, activity, and production of honey particularly in sites that applied pesticides extensively. Many researchers have confirmed toxicity of *A. mellifera* with insecticides focusing in their research on the sub-lethal doses of several chemical groups: parathion-methyl, parathion, carbofuran, diflubenzuron, diazinon, and malathion (Sánchez-Bayo and Goka, 2016; Long and Krupke, 2016; Sánchez-Bayo *et al*., 2017). The acute toxicity of honeybees was demonstrated after exposure to deltamethrin in forages (Decourtye *et al*., 2004).

However, the use of pesticides according to the selectivity in several agro-ecosystems is very important decision to minimize the toxicity and losses in honeybee's population which comes from exposure or contact with pesticides throughout the implementation of certain pest control programs and integrated pest management (IPM). Therefore, the toxicity of sub-lethal dosed to the applied pesticides should be obligatory considered worldwide, but this demand is still required in industrialized nations by European Food Safety Authority (2014), because pesticides are directly threat and impact honeybee's livelihood (Meixner, 2010).

Latterly, intensive studies were directly afforded towards the lethal and sub-lethal toxicity of insecticides on honeybees with significant responses to honeybees in toxicity levels, bumble, and disturbance (Badiou- Bénéteau *et al*., 2013). In Egypt, further studies are needed to the new emerging classes of pesticides to avoid general environmental hazards by chemicals, and particularly towards toxicity on honeybees along with intensive recommendations for its applications.

The selection of pesticides in this study was focused on the using different chemical groups of pesticides targeting several pests in Egyptian agro-ecosystem. Emamectin benzoate as bio-insecticide produced by bacterium Streptomyces avermitilis and works as a chloride channel activator by binding gamma aminobutyric acid (GABAR) receptor and glutamate-gated chloride channels disrupting nerve signals in insects (Grant, 2002). However, imidacloprid (neonicotinoids) interfere with the transmission of stimuli in the insect nervous system and specifically, causes blockage to nicotinergic neuronal pathway by blocking nicotinic acetylcholine receptors (nAChR) (Nauen *et al*., 2001; Tomizawa *et al*., 2007). Chlorpyrifos is an organophosphorus compound which acts by acetylcholinesterase inhibition (Sparks *et al*., 2020) and considered highly effective insecticides, although some are extremely toxic to humans. lambada cyhalothrine (Pyrethroid) shows mode of action in preventing the closure of the voltage-gated sodium channels in the axonal membranes (Soderlund *et al*., 2002). Indoxacarb belongs to the oxadiazine chemical family and is being registered for the control of lepidopterous pests in the larval stages EPA (2000). Insecticidal activity occurs via blockage of the sodium channels in the insect nervous system and the mode of entry is via the stomach and contact routes EPA (2000). The objective of this study is to evaluate the toxicity hazards of selected seven pesticides which are highly recommended to apply in the Egyptian agro-ecosystem on adults of honeybee (workers), *A. mellifera* under laboratory conditions. The selected pesticides were the highly recommended for several pests in Egyptian farms (insect, weeds, and plant fungus). Five pesticides from the seven are insecticides from different groups: (emamectin benzoate, imidacloprid, chlorpyrifos, indoxacarb, lambada cyhalothrine); one herbicide (glyphosate) and the seventh was fungicide (thiophanate-methyl). The acute toxicity for each pesticide was estimated under different exposure period of time such as 24, 48, and 72 hrs.

Material and Methods

The experiments were conducted at the Plant Protection Department, Faculty of Agriculture, Assiut University, Assiut, Egypt.

Honeybee used and hive collection procedures

The local carniolan hybired honeybee workers, *A. melliferea*, was obtained from the apiary of the Plant Protection Department, during the active season of April 2021. The honeybee worker older than 20 days was chosen as the most responsible age class for outside tasks (Winston 1987; Picard-Nizou *et al*., 1995). The careful selection of bee individuals was done according to those free-diseases, and free of previous exposure to any pesticides. The well hygiene hives were selected and smoked two times (30-60s). The forage bees were collected using brushes in plastic bags filled with air and then directly transferred to the lab (34±2 °C and 65±5 %RH) to feed honeybees on sucrose solution.

Pesticides

Pesticides were selected as the most applied by farmers to target several pests in Egyptian agro-ecosystem. Five of them were insecticides: Emamectin benzoate (Avermectin, Basha 1.9%EC), imidacloprid (Neonicotinoid, Midacleed 70%WG), chlorpyrifos (Organophosphorus, Delta Fos 48%EC), indoxacarb (Oxadiazine, Easo plus 30%WG), lambada cyhalothrine (pyrethroid, Lebra 10% EC); one herbicide: glyphosate (Glycine derivative, Roundup star 44.1%SL), and one fungicide: thiophanate-methyl (Thiophanate, Ambition 70%WP).

Toxicity bioassay

The tested cages were made from plastic (volume 350 ml), and each consists of a clear plastic cup with two opening holes: one at the bottom to introduce feeding solution using a 5 ml syringe, and the second side to insert pollen dough using a 2 ml Eppendorf tube that had been opened to make as a dish. To better represent a honeybee in its natural state, a piece of wax comb was added to the cup (Williams *et al*., 2013). The cup's nozzle was covered with an iron grid that let air through (Evans *et al*., 2009).

Two experimental steps were conducted using a combined methodology adapted by protocol of OECD (1998), EPPO (2010), and Medrzycki *et al*. (2013). Preliminary tests were conducted using serial dilutions (1:10) of pesticide stock (1000 ng a.i./L) in distilled water. Seven concentrations were prepared in descending order to identify the dose that gives 10 to 90% mortality.

The oral toxicity tests on forager workers were conducted for 72 hrs. in the lab of each pesticide according to the protocol of OECD (1998). Adults were maintained fast before feeding assay form 1-2 hrs. before initials of the experiment and placed in freezer at 4 °C for one min for easy transition to the plastic cages according to the method of Williams *et al*. (2013). The plastic cages each was enhanced with ten fasted adult bees and kept in incubator (34±2°C and 65±5 % RH). In order to establish a suitable concentration range for each experimental insecticide, several preliminary feeding assays were conducted. The tested concentrations for each pesticide was: (emamectin benzoate: 0.01, 0.05, 0.1, 0.3, 0.5, and 0.8 ppm), (chlorpyrifos: 1.0, 2.5, 5.0 ,10.0 and 50.0 ppm), (imidacloprid 0.1, 1.0, 2.5, 10.0, and 50.0 ppm), (indoxacarb: 1.0, 10.0, 20.0, 50.0 and 100.0 ppm), (lambada cyhalothrine: 25.0, 50.0, 100.0, 200.0, and 500.0 ppm), (glyphosate: 2000.0, 2500.0, 5000.0, 10000.0 and 20000.0 ppm), and (thiophanate-methyl: 100.0, 500.0, 1000.0, 1500.0, and 2000.0 ppm). Each concentration was mixed with sugar syrup before being administered three times for bees were kept in cages with 5 cm syringe. The bee's mortality % was recorded after 24, 48, and 72 hrs. The control treatments were only sugar syrup (1 sugar: 1water). The toxicity experiment of each pesticide was repeated twice, and the percentage of mortality was corrected by Abbott's formula (Abbott, 1925). LC_{50} and slope values of pesticides were determined by Probit regression analysis program and expressed in ppm. The toxicity index calculated according to Sun equations (Sun, 1950):

$$\text{Toxicity index}=\frac{\text{LC50 or LC90 of the most efficient pesticide}}{\text{LC50 or LC90 of the tested pesticide}}\times 100$$

Statistical analysis

Data were subjected to the Probit analysis using the SPSS program version 26 to obtain the LC_{50}, LC_{90}, 95% fiducial limits (FL) and slopes values for these pesticides according to Finney (1971). A significant level of mean separation ($P< 0.05$) was based on non-overlap between the 95% confidence intervals of 2 LC_{50} values and expressed in ppm.

Results and Discussion

Toxicity of pesticides on honeybee workers after exposure periods:

Data in Table 1, 2, and 3 presented different levels of toxicity of the seven tested pesticides on bee workers after 24, 48, and 72 hrs of exposure.

Pesticides toxicity on honeybee workers after 24 hrs

The toxicity of the tested pesticides (Table 1 and Fig. 1 A) stated that, among all tested pesticides emamectin benzoate recorded the most toxic compound with LC_{50} value 0.247 ppm and LC_{90} 5.752 ppm and the toxicity index for both LC_{50} and LC_{90} was 100. On the other hand, glyphosate showed least toxicity level with LC_{50} 6861.151 ppm and, LC_{90} 28243.795 ppm; with toxicity index: 0.003 and 0.020; respectively.

Table 1. Acute toxicity of the tested pesticides on honeybee workers at 24 hr. after exposure

Treatments	Slope ±SE	LC_{50} (ppm) (FL)[1] 95%	Toxicity index (LC_{50})[2]	LC_{90} (ppm) (FL)[1] 95%	Toxicity index (LC_{90})[2]
Emamectin benzoate	0.94±0.09	0.247 (0.113-0.774)a	100	5.752 (1.405-13.119)a	100
Imidacloprid	0.99±0.08	2.437 (1.807 -3.268)b	10.13	47.989 (29.528-91.132)b	11.98
Chlorpyrifos	1.28±0.12	10.226 (8.179- 13.184)c	2.41	101.224 (63.527-193.863)b	5.68
Indoxacarb	1.08±0.11	14.436 (3.635-40.686)c	1.71	220.516 (65.181-28142.285)c	2.60
Lambada cyhalothrine	1.58±0.15	96.967 (17.710-152.824)c	0.254	451.262 (189.598-37744.374)c	1.27
Thiophanate-methyl	1.48±0.15	829.476 (686.901-1002.291)d	0.029	6123.649 (4194.542-10617.521)c	0.093
Glyphosate	2.06±0.18	6861.151 (4629.421-11262.751)e	0.003	28243.795 (15454.753-129623.624)d	0.020

1FL: fiducial limits, 2toxicity 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).

Table 2. Acute toxicity of the tested pesticides on honeybee workers at 48 hr after exposure

Treatments	Slope ±SE	LC_{50} (ppm) (FL) 95%	Toxicity index (LC_{50})	LC_{90} (ppm) (FL) 95%	Toxicity index (LC_{90})
Emamectin benzoate	1.59±0.12	0.047 (0.030-0.067)a	100	0.302 (0.199-0.558)a	100
Imidacloprid	1.47±0.12	0.397 (0.297-0.511)b	11.83	2.949 (2.177-4.316)b	10.24
Chlorpyrifos	2.92±0.25	2.731 (1.765- 3.988)c	1.72	7.496 (4.894-20.543)c	4.028
Indoxacarb	1.35±0.11	5.672 (1.976-10.985)c	0.828	50.542 (24.205-227.898)d	0.597
Lambada cyhalothrine	2.03±0.19	38.504 (15.178-71.492)d	0.122	165.186 (86.172-3789.833)d	0.182
Thiophanate-methyl	1.44±0.14	445.531 (353.015-543.500)e	0.010	3483.234 (2541.645-5400.719)e	0.008
Glyphosate	2.99±0.25	3366.968 (2085.038-4955.121)f	0.0013	9033.695 (5853.760-31656.159)f	0.0033

1FL: fiducial limits, 2toxicity 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).

Pesticides toxicity on honeybee workers after 48 hrs

After 48 hrs of exposure, the toxicity of emamectin benzoate (Table 2 and Fig. 1 B) stills the highest one on honeybee workers after 48 hrs and stayed on the same line of first treatment result with an acute toxicity effect with LC_{50} values 0.047 and LC_{90} 0.302 ppm, and with toxicity index: 100 for both LC_{50} and LC_{90}. Based on the least toxic one, glyphosate still in the same acute level of the previous treatment with LC_{50} value 3366.968 ppm and LC_{90} 9033.695 ppm, with toxicity index: 0.0013 and 0.0033; respectively.

Pesticides toxicity on honeybee workers after 72 hrs:

Data in Table 3 and Fig. 1 C revealed that emamectin benzoate is the highest potent insecticide among all tested groups during the three experimental exposure periods with LC_{50} value 0.020 and LC_{90} 0.072 ppm: with toxicity index 100 for both LC_{50} and LC_{90}. Certainly, glyphosate results showed the lowest toxicity on honeybee workers in all treatments with LC_{50} 2477.276, and LC_{90}= 6203.485 ppm. Generally, the highest slope values (0.938-3.245) for all data revealed that adult honeybee workers were relatively homogenous.

Table 3. Acute toxicity of the tested pesticides to honeybee workers at 72 hr after exposure

Treatments	Slope ± SE	LC_{50} (ppm) (FL) 95%	Toxicity index	LC_{90} (ppm) (FL) 95%	Toxicity index
Emamectin benzoate	2.29±0.20	0.020 (0.016-0.024)a	100	0.072 (0.059-0.094)a	100
Imidacloprid	1.64±0.14	0.329 (0.250-0.421)b	6.079	2.003 (1.507-2.853)b	3.594
Indoxacarb	1.41±0.12	1.985 (1.389-2.659)c	1.007	16.082 (12.092-22.695)c	0.447
Chlorpyrifos	3.25±0.28	2.086 (1.465-2.764)c	0.958	5.179 (3.745-9.625)c	1.39
Lambada cyhalothrine	2.49±0.28	25.029 (5.400-39.758)d	0.079	81.597 (51.779-333.918)d	0.088
Thiophanate-methyl	1.59±0.14	367.229 (292.655-444.749)e	0.005	2340.841 (1809.520-3290.977)e	0.003
Glyphosate	3.22±0.32	2477.276 (2187.102-2754.116)f	0.0008	6203.485 (5352-7597.831)f	0.001

1FL: fiducial limits, 2toxicity 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).

Honeybee workers are on the frontlines of exposure to pesticides. In this interim, we evaluated the toxicity of seven pesticides which are highly recommended to apply in the Egyptian agro-ecosystem on adults of honeybee workers, *A. mellifera* under laboratory conditions. There was a little difference in the toxicity levels among the rest of selected pesticides in the three exposure hours of treatments. In the first 24 and 48 hrs the acute toxicity of pesticides was ordered in the same line from the most to the least toxic compounds as followed: emamectin benzoate, imidacloprid, chlorpyrifos, indoxacarb, lambada cyhalothrine, thiophanate-methyl, and glyphosate. Meanwhile, this order showed slight changed in the last treatment exposure period of 72 hrs that indoxacarb recorded the highest toxicity with LC_{50} value 1.985 ppm and LC_{90} 16.082 ppm than chlorpyrifos with LC_{50} value 2.086 ppm and LC_{90} 5.179 ppm.

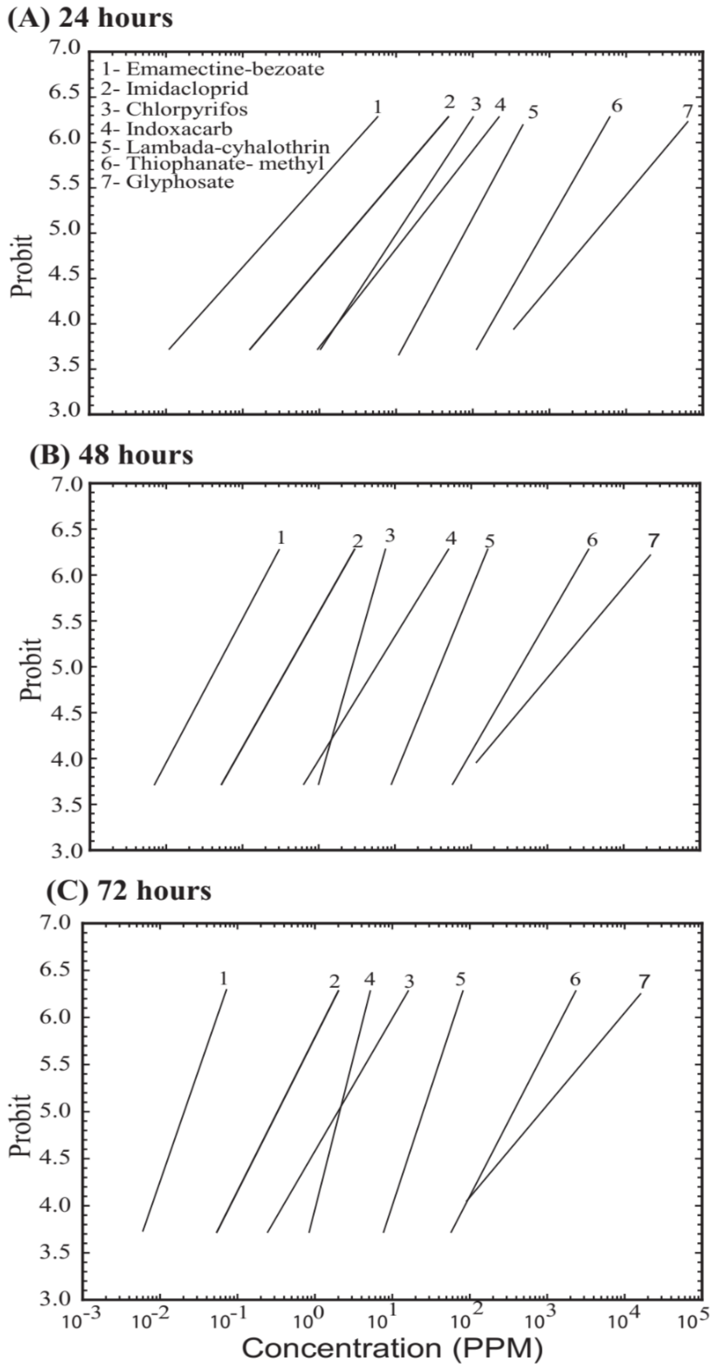

Fig.1. Toxicity (LC_{50}) of oral concentration mortality response curve of seven pesticides on honeybee, *A. mellifera* workers at different exposure time (24, 48, and 72 hrs).

These results indicated that emamectin benzoate was highly toxic to honeybee workers after 24, 48 and 72 hrs of exposure. Similar trend was also found by Ghasemi *et al*. (2022), which indicated that, worker bees were treated with the field recommended concentration of emamectin benzoate with three routes of exposure including residual contact, oral, and spray within the laboratory. They reported that, based on estimated median survival times (MSTs), emamectin benzoate was highly toxic to the bees, especially when applied as spray. Abdu-

Allah and Pittendrigh (2018) indicated that emamectin benzoate as orally 3.3, 7.6, and 31.7-fold and topically 133.3, 750.0, and 38.3-fold more toxic than abamectin, spinetoram and spinosad, respectively. They stated that the high contact toxicity of emamectin benzoate compared to its analogue, abamectin, may be due to higher penetration and/or lower metabolic detoxification a hypothesis that remains to be tested. In addition, avermectins have high absorption coefficients, and the results here are in keeping with several other similar investigations with bees and other insects (Bloom and Matheson 1993; Gupta *et al*., 2005; Lumaret *et al*., 2012; Abdu-Allah and Pittendrigh, 2018).

Importantly, imidacloprid was the 2nd toxic insecticide for the honeybee workers after 24, 48 and 72 hrs of exposure with LC_{50} values 2.437, 0.397 and 0.329 ppm, respectively. The lethal dose/concentration of imidacloprid in the honeybee, *A. mellifera* has been widely surveyed, and the levels vary among different regions and seasons. These results were partial agreement with Abbassy *et al*. (2020) how stated that, the LC_{50} (oral application) under laboratory conditions is 3 ppb at 24 h and 0.6 ppb at 48 h, while the LD_{50} (topical application) is 29 and 26 ng/bee at 24 and 48 h, respectively. Compared to adults, honeybee larvae can tolerate higher dosages of imidacloprid at LD_{50} = 4.17 µg and LC_{50} = 138.84 ppm (Dai *et al*., 2017). Laurino *et al*. (2013) reported that, the acute oral toxicity LD_{50} value for imidacloprid at 24 h is 118.74 ng/honeybee, and at 48 and 72 h, it is 90.09 and 69.68 ng/bee, respectively.

The present study demonstrated that chlorpyrifos was the 3rd toxic pesticides on honeybee workers under laboratory conditions with LC_{50} values 10.226, 2.731 and 2.086 ppm after 24, 48 and 72 hrs of exposure, respectively. Previous studies have demonstrated a high acute oral toxicity of chlorpyrifos to honeybees (Potts *et al*., 2010; Johnson *et al*., 2010; Sanchez-Bayo and Goka, 2014; Dai *et al*., 2019). Chlorpyrifos was a relatively high toxicity to bees compared to other pesticides and sublethal doses may threaten the success and survival of honeybees (Dai *et al*., 2019). This result explains that organophosphorus showed some degradation for a longer time in the environment than carbamate.

Our findings about indoxacarb are agreed with the report of EPA (2000) which showed that indoxacarb is "practically non-toxic" by dietary intake and "highly toxic" by contact for honeybee. Previous study by Bonmatin *et al*. (2015) and Pashte and Patil (2018) indicated that, indoxacarb was the most toxic compound by direct contact to honeybee *A. mellifera*, compared with the pyrethroid cypermethrin and the neonicotinoid imidacloprid. The toxicity of indoxacarb as observed in the present investigation is in contrast with Zhu *et al*. (2015) who found that the LD_{50} value was 1.80µg/bee and LC_{50} value of 1140 mg/L, for formulated indoxacarb to *Apis mellifera*. Also, the obtained LC_{50} value for indoxacarb is differ from that reported by Yu *et al*. (2009), which was 3.54 mg/L. By contrast, Abbassy *et al*. (2020) reported that indoxacarb was the most toxic by contact method for honeybee with LD_{50} values 0.0018 µg per bee.

The lower oral toxicity of glyphosate (herbicide) and thiophanate methyl (fungicide) on honeybee may have resulted from low water solubility or from high enzymatic metabolic activity, or a combination, therefore the results in only low amounts of poison to the insecticide site of action. Johnson (2015) similarly found fungicides are usually not considered acutely toxic to bees. Long and Krupke (2016) and Tsvetkov *et al*. (2017) found that some active ingredients of fungicides with low toxicity may pose chronic risks to bees if they are repeatedly present in the environment throughout the season. There is recent evidence of both lethal and sublethal impacts following exposure to some widely used fungicides from studies of both honeybees and wild bees (Cullen *et al*., 2019; Belsky and Joshi, 2020). Herbicides such as glyphosate are also known for harming bees either directly through oral or contact exposure (Sharma *et al*., 2018) or indirectly by reducing the diversity and abundance of flowering plants (or weeds) which are important food resources for insect pollinators (Bretagnolle and Gaba, 2015).

In conclusion, the toxicity of pesticides on honeybee workers depends on several factors, For instance, the type of pesticide, the dose, and the exposure time. Some pesticides are more toxic than others although low doses can have harmful effects on bees. All the tested insecticides were toxic to honeybees, *A. mellifera*, based on laboratory results when employed in feeding methods, with the exception of glyphosate and thiophanate methyl. Additionally, to reduce the toxicity of pesticides on honeybee workers. It is important to use IPM practices that minimize the use of pesticides and promote alternative methods of pest control. It is also important to follow label instructions carefully when using pesticides and avoid applying them during times when bees are most active especially during flowering periods.

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تأثير سمية بعض مبيدات الآفات على نحل العسل ***Apis melliferea*** **L. تحت ظروف المعمل**

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الملخص

يتعرض نحل العسل عادة لمبيدات الآفات بشكل مباشر أو غير مباشر من خلال البحث عن الغذاء. في هذه الدراسة المعملية تم اختبار تأثير سمية سبع مبيدات افات تم اختبارها من مجموعات مختلفة من المبيدات الزراعية التي يشيع استخدامها: إيمامكتين بنزوات، إيميداكلوبريد، كلوربيريفوس، إندوكساكارب، لامبادا سيهالوثرين، جليفوسات وثيوفانات ميثيل على شغالات نحل العسل خلال فترات تعرض مختلفة بعد 72،48،24ساعة .تم ترتيب المبيدات المختارة حسب سميتها من الأكثر إلى الأقل سميه على النحو التالي: إيمامكتين بنزوات، إيميداكلوبريد، كلوربيريفوس، إندوكساكارب، ولامدا سيهالوثرين وفي الوقت نفسه، كان ثيوفانات - ميثيل وجليفوسات أقل سمية بين مبيدات الآفات المختارة .كان أعلى مبيد سمية خلال فترة التعرض 24و48و72 ساعة هو إيمامكتين بنزوات بناءً على قيم التركيز النصف مميت0.047،0.247و0.020جزء في المليون) والقيم المسببة لموت 90% 0.302،5.752و0.072جزء في المليون) على التوالي، وكان أقل مبيد هو مبيد الحشائش الجليفوسات مع LC_{50} قيم (6861.151و3366.968 و2477.267 جزء في المليون) وقيم LC_{90} تبلغ (28243.795 و9033.695 و6203.485 جزء في المليون) بعد 24 و48 و72 ساعة من التعرض على التوالي .ومع ذلك، سجل الكلوربيريفوس الثالث في الترتيب للمبيدات المختبرة عند 24 و48 ساعة من التعرض بقيم LC_{50} تبلغ 10.226 و2.731 جزء في المليون وقيم LC_{90} تبلغ (101.224و7.496 جزء في المليون لمدة 24و48 ساعة على التوالي)، بينما بعد 72 ساعة سجل المبيد السام الرابع بناءا على قيم 2.086LC_{50} و 5.179LC_{90} جزء في المليون وبناءً على مؤشر السمية، أوضحت النتائج أنه تم ترتيب أكثر المبيدات سمية على النحو التالي: إيمامكتين بنزوات>إيميداكلوبريد>كلوربيريفوس>إندوكساكارب>لامبادا سيهالوثرين>ثيوفانات-ميثيل>جليفوسات .أظهرت هذه النتائج أن مبيدات الآفات شديدة السمية على شغالات نحل العسل لذا يجب عدم استخدامها في الأوقات التي يكون فيها النحل أكثر نشاطًا مثل فترات الإزهار.