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Selective Toxicity of Neonicotinoids Compounds against *Apis mellifera* Workers

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ABSTRACT

Honeybee is an important economic insect provide man with honey product give a good service by increasing crop production with his crop pollination services .Neonicotinoids as Acetamprid ,Thianethoxam and Clothianidin introduced to the environment to control different types of pests attack cultivated crops.Oral and indirect contact trails were carried out on adult worker honey bees for each pesticide, using commercial formulations. The acute oral toxicity (LD_{50})and the acute indirect contact toxicity (LC_{50}) were calculated Mean LD_{50} values and LC_{50} values at 24 hrs for Acetamiprid was $114.72x10^3$ ng/bee , $1.58x10^5$ ppm , For Thiamethoxam was 740 ng/bee, $o.15x10^4$ppm and for Clothianidin 330ng/bee, $8.8x10^2$ppm respectively. compared with traditional organophosphate Dimethoate LD_{50} 120 ng/bee ,and LC_{50} $3.4x10^4$ppm . The data shows the same pattern after 48 and 72 hours of treatment. The neonicotinoids compounds are more safe to use against bees under laboratory condition at different time intervals compared with Dimethoate Organophoshate compound.. The safety margin for Acetamiprid LD_{50} was 952, 917 and 984 after 24, 48 and 72 hours of application compared with dimethoate LD_{50} , however due to LC_{50} was 465, 836 and 592 times dimethoate toxicity. Acetamiprid was much safe more than Thiamethoxam than clothianidin.Neonicotinoids compound shows that its more safe to use in the environment while bees active or close to treated plants.

INTRODUCTION

Honey bee may be raised because of its economic importance in many products, the most important of which is the production of honey and Wax ،honeybee rely on flower plants while foraging and collecting its food sources of nectar and pollen then it is one of the important groups that act as pollinators for a large number of crops) Sandrock *et al* ،2014 Cresswell (2011 Regret that honey bees are always exposed to pollution of various environmental pollutants. But the sub lethal exposure to widespread agricultural pesticides may also affect bees) sandrock *et al* . (2014 ؛Therefore, there is a great concern about the declineof the honey bee population) *Apis mellifera* (in several parts of the world mainly due to improper application of insecticides (Matsumoto,2013.(In the last 20 years, pesticide use has shifted away from OPS and carbamates toward neonicotinoides compounds that are agonists of insect nicotinic) AChEs(receptors (Elbert *el al*.(2008 ؛Also demonstrated that exposure to sub lethal doses of combined cholinergic pesticides significantly impairs important behavior involved in foraging ,implying that pollinator population decline could be the result of a failure of neural function of bees exposed pesticide in agricultural landscapes) .Williamson and Wright .(2013،Moreover exposure to pesticides has produced negative effects on individual bees and their colonies for nearly a century).Hassona and Kordy (2014، As neonicotinoids are strongly suggested to be systemic .)Aliouane *et al* .(2009؛Objective of the study to determine the effect of three neonicotinoide on bees in direct (LD) or indirect (LC) effect comparative with a traditional O.Pdimethoate compound.

MATERIALS AND METHODS

Apis melliphera workers used in this study have been provided from honey been Colony reared in El-Sabahia stations ، Abis ، Alexandria .Honey bees workers was in adult and weight 0.1 gm. /bee Formulated pesticides have been used in this study are Thiamethotam (Actara 20%W.G), Clothianidin (Super Tox-1 48%S.C), Acetamiprid (Setar 20% S.P) and Organophosphate (Dimethoate 40%E.C). All pesticidsides used are in formulated form. A stock solution of each compound was prepared freshly in water solution.

Insecticide concentrations were applied to honey bees workers by surface treatment application method by impregnated 9 cm diameter Whitman No 1 round filter paper with 1 ml volume of pesticide concentration and hold until dryness. Transfer treated filter paper into petri dish have 10 honeybees worker and four replicate were made for each concentration .Also four petri dishes were used as control have filter paper treated with water only . Mortality were recorded after 24, 48 and 72hours of application. Calculated Lethal concentration LC_{50}value, confidence limits, and slope values of the regression lines were done according to the method of Litchfield and Wilcoxon (1949) .

Topical application was used for measuring direct susceptibility level for honey bees. This was carried out as fellows. Arnold Hand Micro Applicator – barkad Manufacturing company Limited, England . This applicator is developed to give a range from 0.25 to 0.5 ml. one or two micro liter of insecticides concentration were applied topically to the thorax honey bee workers treated insects transferred to a petri dish covered by glass plate . Ten insect used for each plate and four replicate were used for each concentration. Four plates were used as control insects were recorded with water only. Mortality was recorded after 24, 48, and 72 hours. The mortality lines and LD_{50}so values calculated according to Litchfield and Wilcoxon (1949).

RESULTS AND DISCUSSION

Data presented in Table (1) show the toxicity pattern of Acetamiprid) Setar % 20 S.P ،(Thianethoxam) Actara %20W.G ،(Clothianodin) Super tox-1 S.C 48 %), and Dimethoate % 40 E.C against honey bees workers in direct (Topical application (and indirect (Surface application) effect. The data show that traditional organophosphate dimethoate compound was the most toxic tested compound against honey bees workers under study with LC_{50}concentration equal 3400, 140 and 110 ppm after 24 , 48 and 72 hours of treatment and with acute oral toxic effect LD_{50}concentration equal 120 , 71 and 45 ng/bee after 24 , 48 and 72 hours of treatment. Oral and indirect contact trials were carried out on adult workers of honey bees for each one of three commercial formulation of neonicotinoid insecticides .The acute oral toxicity (LD ($_{50}$was 114.27x10 ،^{3} 65.17x10 ^{3}and 44.28x10 ^{3}ng/bee for Acetamiprid after 24 , 48 and 72 hours of application., However it was 740, 320 and 150 ng/bee for Thiamethoxam after 24, 48 and 72 hours of application however it was 330, 170 and 130 ng/bee for clothianidin after the three interval times under study 24, 48 and 72 hours. On the other hand the the acute indirect contact toxicity (LC ($_{50}$was 1.58x1.17 ، 10^{5}x10 ^{5}and0.65 x10 ^{5}ppm concentration for Acetamiprid after 24 , 48 and 72 hours of application ،. Hoowever it was0.15 x100.1 ،^{4}x10 ^{4}and0.03 x10 ^{4}ppm for Thiamethoxam after 24, 48 and 72 hours of application however it was 8.8x103.1 ،^{2}x10 ^{2}and 0.84x10 ^{2}ppm for clothianidin after the three interval times under study 24, 48 and 72 hours ،this which agree with which found before by) Palmer *et al*2013 and Decourtye *et al*.(2005 Acetamiprid (Setar 20 % S.P) was the most safe compound with LC_{50}1.58x10^5 , 1.17x10^5 and 0.65x10^5 ppm concentration against honey bees workers after 24 , 48 and 72 hours of oral indirect treatment however LD_{50} values (The acute oral toxicity) were 114.27x10^3, 65.17 x10^3 and 44.28 x10^3 ng/bee after 24 , 48 and 72 hours of topical application treatment (Thomazoni *et al* ;2009). This data clear that Acetamiprid compound was the most safe compound to bees in the environment followed by thiamethoxam and the least one was clothianidin, .This data follow the same pattern found before by others(Laurino *et al* 2011, Laurino *et al* 2013,and Oliveira *et al* 2014).

Table 1. Toxicity of three neonicotinoid compounds and dimethoate against honey bees worker using two methods of applications at three interval time

Tested Compound	LC_{50} (ppm) 24hrs	48hrs	72hrs	LD_{50} (ng /bee) 24hrs	48hrs	72hrs
Acetamiprid	$1.58x10^5$	$1.17x10^5$	$.65x10^5$	$114.27x10^3$	$65.17x10^3$	$44.28x10^3$
Slope	0.784	0.632	0.727	0.807	0.723	0.667
Thiamethoxam	$0.15x10^4$	$0.1\ x10^4$	$.03x10^4$	740	320	150
Slope	0.553	0.515	0.395	0.753	0.657	0.593
Clothianidin	$8.8\ x10^2$	$3.1\ x10^2$	$.84x10^2$	330	170	130
Slope	0.388	0.379	0.338	0.617	0.602	0.732
Dimethoate	$3.4\ x10^4$	$1.4\ x10^2$	$1.1x10^2$	120	71	45
Slope	0.715	0.706	0.788	0.686	0.661	0.66

Table 2. Estimated the toxicity rated of three tested Neonicotinoids against honey bees workers comparing with traditional insecticide Dimethoate toxicity at three time intervals of effect and two application methods.

Tested Compound	Time hrs	LC_{50} Toxicity (ppm)	Ratio	LD_{50} Toxicity (ng /bee)	Ratio
Acetamiprid	24	$1.58\ x10^5$	465.94	114.2710^3	952.25
	48	$1.17\ x10^5$	836.85	65.1710^3	917.88
	72	$0.65\ x10^5$	592.81	44.2810^3	984.00
Thiamethoxam	24	$1.51\ x10^3$	4.44	740	6.166
	48	$1.0\ x10^3$	7.14	340	4.788
	72	$3.2\ x10^3$	2.909	150	3.33
Clothianidin	24	$8.8\ x10^2$	2.58	330	2.75
	48	$3.1\ x10^2$	2.21	170	2.39
	72	$0.84\ x10^2$	0.763	130	2.88
Dimethoate	24	$3.4\ x10^2$	1.00	120	1.00
	48	$1.4\ x10^2$	1.00	71	1.00
	72	$1.1\ x10^2$	1.00	45	1.00

The data in Table (2) cleared that acetamiprid compound was much more safe in the environment to control different types of pests without harm honey bees with safe ratio 952 times less than dimethoate toxicity and in LD_{50} was 465 times less than dimetoate toxicity (Brunet *et al* ;2005).

The data presented can emphasize that acetameprid (setar 20% SP), has acute oral toxicity values were quite similar to and in the same order of magnitude of the data reported by (Toomlin 2003, Decourtye and Devillers 2010). These contrasting result were likely due to the particular features of acetamiprid toxicology and its non-sigmoidal dose effect relationship (Suchail *et al* 2000), .Surely methodologyical shortcomings of the oral toxicity bioassay , like the ingestion of unequal doses or neutrative status of honey bees at time of application (Nauen *et al*; 2001) could be taken in consideration but they should have occurred also with other neonicotinoids

Thiamethoxam and Clothianidin LC_{50} values and LD_{50} values at different interval times 24 , 48 and 72 hours of application were markedly lower than obtained with Acetamiprid, which agree with which found before by (Bailey *et at* 2005 and Sgolastra *et al* 2012). The data cleared that acetamiprid is much safe to use for controlling different types of field pests in the presence of bees colons .But this situation cannot be used with all neonicotinoids because each compound behave toxicologically in special pattern different than other.

Our data is answer the most important question about the safe margin which we can control pests on crops during the active period of honey bees workers in the environment to reach the most safe and selective compound which we can use.

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السمية الاختيارية لمركبات النيونيكوتينويد على شغالات نحل العسل ***mellifera Apis***

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نحل العسل من الحشرات الاقتصاديه الهامه للأنسان و التي تعطي خدمه جيده بزياده انتاج المحاصيل الحقلية. النيونيكتينويدز Neonecotinoids ومنها الاسيتامبريد، السياموزوكسام، كلوثيانيدين التي ادخلت علي البيئه لمكافحة انواع مختلفه من الافات التي تهاجم المحاصيل الزراعيه. اجريت التجارب بمعامله شغالات نحل العسل مباشرة بالمعاملة السطحية للحشرة وغير مباشر بمعاملة الاسطح باستجدام التركيبات التجاريه. قدرت قيم LD_{50} و LC_{50} للاسيتامبريد بعد 24ساعه فكانت 114,72 x 10^{3} ، 1,58 x 10^{5} جزء فى المليون ، وللسياموزوكسام 740 نانو جرام / نحلة و 0,15 x 10^{4} وللكلوثيانيدين 330 نانو جرام / نحلة 8,8 x 10^{2}جزء فى المليون على التوالى اما الاورجانوفوسفات دايمثويت LD_{50} 120 نانو جرام / نحلة ، LC_{50} 3,4 x 10^{4} جزء فى المليون . تظهر البيانات بنفس النمط بعد 48 – 72 ساعة من المعاملة . مركبات Neonecotinoids تكون اكثر امان فى الاستخدام ضد النحل تحت ظروف المعمل مع فترات زمنية مختلفة بالمقارنة بالدايمثويت وكان التطبيق امن للاسيتامبريدLD_{50} 952 ، 917 ، 984 ، بعد 24 – 48 – 72 ساعة من التطبيق بالمقارنة بالدايمثويت LD_{50} ، بينما نتيجة الـ LC_{50} كانت 465 ، 836 ، 592 مرة لسمية الدايمثويت . الاسيتامبريد كان اكثر امان من السياموزوكسام،كلوثيانيدين . تظهرمركبات Neonecotinoids انها ذات اختيارية عالية و اكثر امان فى الاستخدام البيئي اثناء نشاط النحل او معاملة الافات الموجودة على المحاصيل المختلفة.