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ORIGINAL RESEARCH ARTICLE

The effect of using imidacloprid and chlorpyrifos and their nanoforms on certain characteristics of honeybee *Apis mellifera* L.

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Abstract

This study was conducted in the apiary of Faculty of Agriculture, Cairo University, Egypt, to examine and determine the effect of Imidacloprid & Chlorpyrifos and their nano compounds on certain morphological characteristics of honeybee workers. This study proved that: Chlorpyrifos and nano-chlorpyrifos compounds showed more toxicity and harmful than Imidacloprid and nano-imidacloprid towards honeybee workers, especially on body balling extent and mouthparts out & straight by percentages 80.27% and 78.71% for nano-chlorpyrifos and chlorpyrifos, respectively. Imidacloprid and its nano form had the same effect approximately for body balling, legs shrinkage and abdomen length. In contrast, nano-imidacloprid recorded the lowest damage on honeybee wings horizontality and mouthparts out & straight by percentages 46.40% and 16.92%, respectively. Also, only nano-chlorpyrifos showed negative and significantly effect on abdomen length of honeybee worker by value 0.48 cm.

Keywords Honeybee · Pollinators · Imidacloprid · Chlorpyrifos · Nano · Insecticide

Introduction

Honeybee "*Apis mellifera*" is considered one of the most important pollinators for many crops in the world. As well as, it has a tangible impact by produce many products such as honey, royal jelly, pollen, wax and propolis. Also, maintaining the ecosystem balance (Suchail et al. 2000; El-Masarawy 2010). But, on the other hand the over and unjust using of different types of pesticides makes an evident decrease of bee density (McLaughlin and Mineau 1995; Potts et al. 2010; Van Dijk et al. 2013). So, this led to occurrence unusual phenomenon called colony collapse disorder or "colony losses" (Neumann and Carreck 2010). Many reasons caused to this phenomenon, pesticides are considered one of them (Belzunces et al. 2012; Sandrock et al. 2014).

Imidacloprid (1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine) is a remarkable insecticide versus many pests, especially sucking ones, and it considered the most commonly compound used from chloronicotinyl group, it's exists as a nitroguanidine systemic molecule. Mode of action of imidacloprid is directly effect on the nicotinic acetylcholine receptors of insects (Buckingham et al. 1997; Bicker 1999). Also, Imidacloprid used by a widely range scale like seed dressing and spraying. However, the negative side effects of these applications showed harmful and toxic effect for honeybee workers, those poisoned either with the original compound or metabolite compounds (Suchail et al. 2000). The second compound in this study was chlorpyrifos, it's an organophosphorus insecticide, it widely used for soil-borne insect pests on a lot of many crops (Solomon et al. 2014). Organophosphorus insecticides (OPs) are also one of the most widely used classes of insecticides. They act on the nervous system of insects by inhibiting acetylcholinesterase. Residues of OPs have been detected in colony matrices (Mullin et al. 2010) and their potential hazard to colonies has been noted (Cutler et al. 2014; Al Naggar et al. 2015). Chlorpyrifos is an OP pesticide used foliarly in crop management (Solomon et al. 2014). It has a relatively high toxicity to bees compared to other pesticides (Johnson et al. 2010) and sublethal doses may threaten the success and survival of honey bees. Recently, more interest and a lot of reports suggested that using nano forms of pesticides as an alternative tool for pest control. However,

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nano-imidacloprid has been used to control variety species of pests (Assemi et al. 2014).This study aim to examine the effect of imidacloprid & chlorpyrifos and their nano forms on five morphological parameters of honeybee workers.

Materials and methods

This study was carried out in the apiary of Faculty of Agriculture, Cairo University, Egypt, during spring 2019. Two pesticides compounds were used (Imidacloprid and Chlorpyrifos) and their nano forms in this study.

Nano pesticides were prepared in Nano Tech. company located in Dreamland, 6th October city, Egypt, according to the technique described by Guan et al. (2008). Fifteen workers of honeybee *Apis mellifera* L. were selected from suitable hives in the apiary for each replicate, forty five workers for each concentration of previous compounds. Also, three concentrations were examined under this study 100, 200 and 300 ppm/each compound.

Five parameters which taken into consideration, body balling, legs shrinkage, wing horizontality, abdomen shortage and mouthparts out & straight. All of these parameters were measured in laboratory under temperature 23 ± 2°c and 65 ± 5% relative humidity, by using equipped wooden boxes (3 boxes/ 3 concentrations/compound), the width of each box is 75 cm. & 80 cm. height, and enclosed by perforated wire mesh for all sides.

Each box was supplied with one petri dish 10 cm. diameter, it contained 10 ml. of insecticide plus sugar syrup (water: sugar is 1:1). In addition that one box separately as a control replicate without any pesticide, only sugar syrup was added.

The results in the study were analyzed by using Analysis of Variance (ANOVA) in a randomized complete block design (RCBD) using MSTAT-4C (1989) computer program.

Table 1 The effect of four insecticides on some characteristics of honeybee workers

Insecticide	Conc. (ppm)	Parameter				
		% Body Balling	% Legs Shrinkage	% Wing Horizontality	Abdomen Shortage (cm.)	% Mouthparts out & Straight
Imidacloprid	100	65.47*ab*	73.80*a*	77.37*ab*	0.57*a*	40.80*cde*
	200	50.60*b*	55.33*ab*	74.50*abc*	0.54*ab*	18.47*ef*
	300	68.33*ab*	25.00*b*	53.33*abc*	0.46 *cd*	78.33*ab*
Mean		**61.47*a***	**51.38*a***	**68.40*a***	**0.52*a***	**45.87*b***
Nano-Imidacloprid	100	55.40*b*	63.10*ab*	50.30*bc*	0.55*ab*	31.87*def*
	200	88.90*ab*	66.67*ab*	38.90*c*	0.51*bc*	5.57*f*
	300	53.33*b*	26.67*b*	50.00*bc*	0.51*b*	13.33*f*
Mean		**65.88*a***	**52.14*a***	**46.40*b***	**0.52*a***	**16.92*c***
Chlorpyrifos	100	69.33*ab*	69.33*ab*	51.40*bc*	0.57*a*	47.23 *cd*
	200	77.67*ab*	66.57*ab*	88.90*a*	0.53*ab*	100.00*a*
	300	88.67*ab*	77.33*a*	44.43*bc*	0.44*d*	88.90*ab*
Mean		**78.56*a***	**71.08*a***	**61.58*ab***	**0.51*A***	**78.71*A***
Nano-Chlorpyrifos	100	80.57*ab*	61.10*ab*	51.40*bc*	0.46 *cd*	75.00*ab*
	200	65.00*ab*	41.67*ab*	66.67*abc*	0.53*ab*	83.33*ab*
	300	95.23*a*	58.73*ab*	38.10*c*	0.46 *cd*	63.10*bc*
Mean		**80.27*a***	**53.83*a***	**52.06*ab***	**0.48*b***	**73.81*a***
Conc./ insecticides/parameter	100	67.69	66.83	57.62	0.54	48.73
	200	70.54	57.56	67.24	0.53	51.84
	300	76.39	46.93	46.47	0.47	60.92
Mean		**65.47**	**73.80**	**77.37**	**0.57**	**40.80**
L.S.D at 5%						
Insecticide		**NS**	**NS**	**21.61**	**0.03021**	**15.68**
Concentration		**NS**	**NS**	**18.71**	**0.02616**	**NS**
Insectcide x Conc.		**39.42**	**45.32**	**37.43**	**0.05233**	**27.15**

Means in the same column followed by the same letters are not significantly different at 5% level of significance

Results

Collecting data was continued from all replicates, all died bees each six hours were collected and transferred into clean petri dish, and examined for certain following mentioned parameters.

Body balling

Data tabulated in Table 1 and Fig. 1 showed that chlorpyrifos and nano-chlorpyrifos have highly effect on the degree of body balling in honeybee workers than another two types of insecticides with non-significant variance between them.

The highest effect came from 300 ppm concentration of nano-chlorpyrifos with percentage 95.23%, followed by the second concentration of nano-imidacloprid then third concentration of chlorpyrifos with percentages 88.90% and 88.67, respectively. Whereas, the lowest effect of pesticides was recorded with concentration 200 ppm for imidacloprid by percentage 50.60%.

Legs shrinkage

On the same trend approximately, data of this parameter went, particularly with chlorpyrifos. Data in Fig. 2 showed that 300 ppm concentration of chlorpyrifos and 100 ppm concentration of imidacloprid made the highest pecentages of legs shrinkage of honeybee workers by 77.3% and 73.8%, respectively. On the contrary, results showed the lowest effect on legs shrinkage came from the third concentration (300 ppm) of both nano-imidacloprid and imidacloprid with percentages 26.7% and 25.0%, respectively. Moreover, chlorpyrifos made a major effect on legs shrinkage with mean percentage 71.1%. Generally, no significant variance occurred between all treatments as shown as Table 1.

Wing horizontality

Contrary to what mentioned of parameters above, highly significant variance occurred between imidacloprid and nano-imidacloprid by general means 68.40% and 46.40%, respectively as shown as Table 1. Moreover, results illustrated in Fig. 3 clear that the second concentration of chlorpyrifos (200 ppm) made the largest effect on wing horizontality of honeybee workers with percentage 88.90%. Whereas, nano-imidacloprid (200 ppm) and nano-chlorpyrifos (300 ppm) showed the lowest percentages in this parameter with values 38.9% and 38.1%, respectively.

Abdomen shortage

Results in Fig. 4 presented that on the whole, the negative effect of nano-chlorpyrifos on abdomen shortage parameter was the highest one, where the length of abdomen reached to 0.48 cm, compare with another pesticides were recorded 0.51 cm, 0.52 cm and 0.52 cm for chlorpyrifos, imidacloprid and nano-imidacloprid, respectively.

Besides, the largest and lowest decrease in abdomen length happened by 300 ppm concentration of chlorpyrifos and 100 ppm concentration of imidacloprid when abdomen length reached to 0.44 cm and 0.57, respectively Table 1.

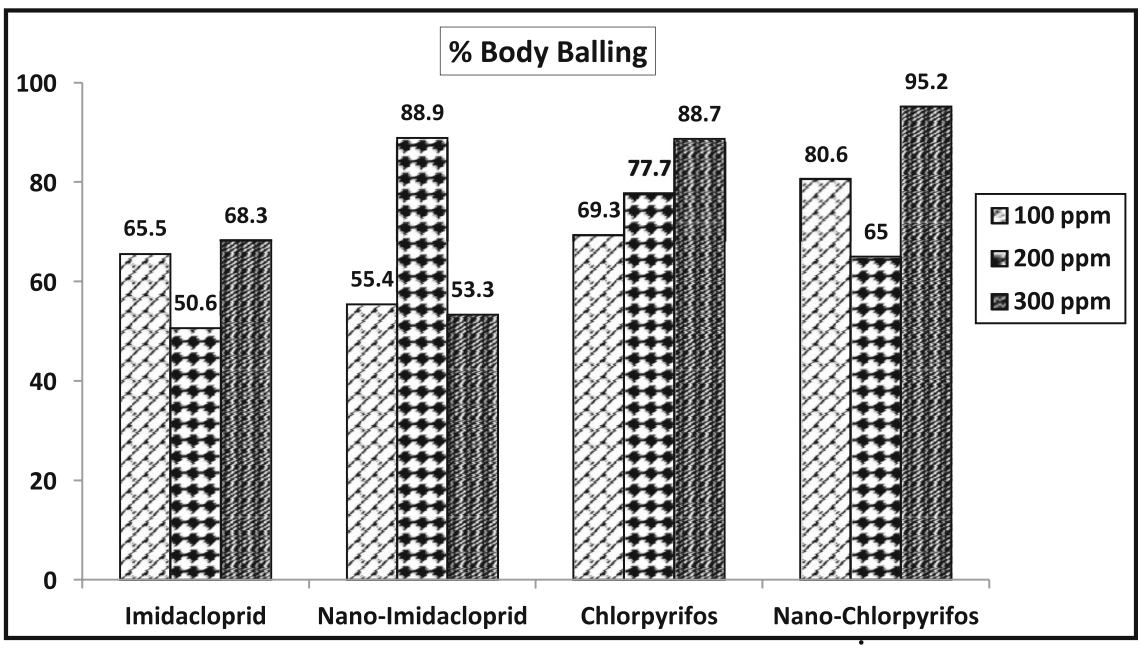

Fig. 1 Effect of three concentrations/insecticide (%) on body balling of honeybee worker

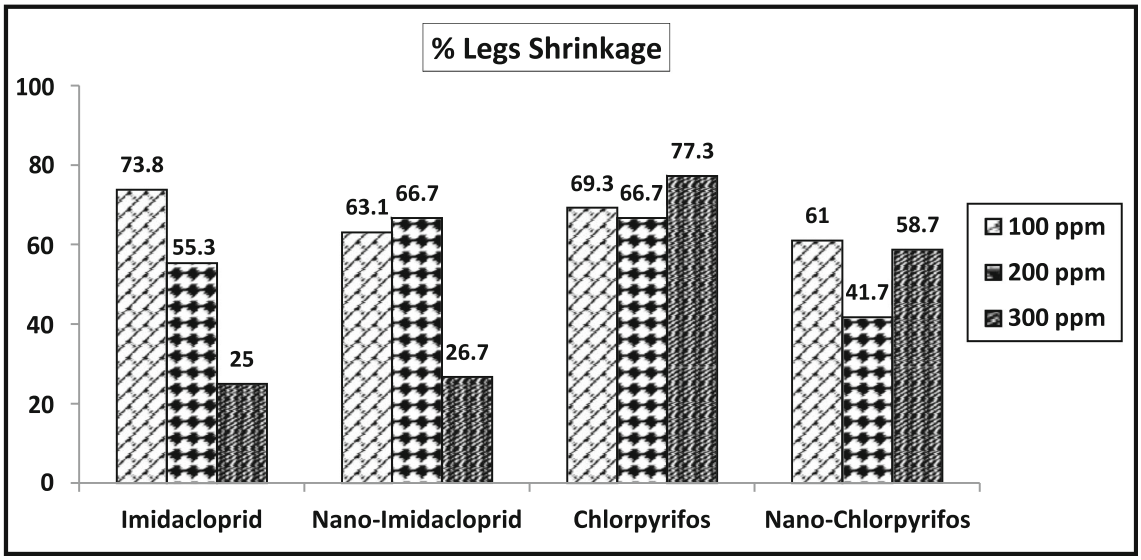

Fig. 2 Effect of three concentrations/insecticide (%) on legs shrinkage of honeybee worker

Mouthparts out & straight

Results in Table 1 and Fig. 5 clear that the lowest damage on mouthparts occurred by using nano-imidacloprid with percentage 16.92%, followed by imidacloprid with percentage 45.87%. Also, highly significance between imidacloprid and nano-imidacloprid. In opposite, large harmful happened with chlorpyrifos and nano-chlorpyrifos reached to 78.71% and 73.81%, respectively.

Discussion

Chlorpyrifos (CPF) is a broad-spectrum organophosphate pesticide that is used heavily throughout the world for agriculture and domestic purposes it has been widely used for decades to control pests several yeildes (Abd El-Fattaha et al. 2019). Nano forms of this pesticide used recently to control red palm weeivel successfully in both field and laboratory (Abd El-Fattaha et al. 2019, 2020).

Imidacloprid showed unique toxicological characteristics, where delayed of mortality kinetics when doses increased. Also, mortality occurred after 4 h of acute intoxication. Nevertheless, neurotoxicity symptoms and abnormal behavior appeared within 10 min. Following intoxication, e.g. nervous, trembling and downfall. However, perhaps mortality elongated to more than 96 h. (Suchail et al. 2000, 2001; Sánchez-Bayo et al. 2017).

Decourtye et al. (2003) studied sublethal concentrations of imidacloprid and metabolite, 5-OH imidacloprid, on caged

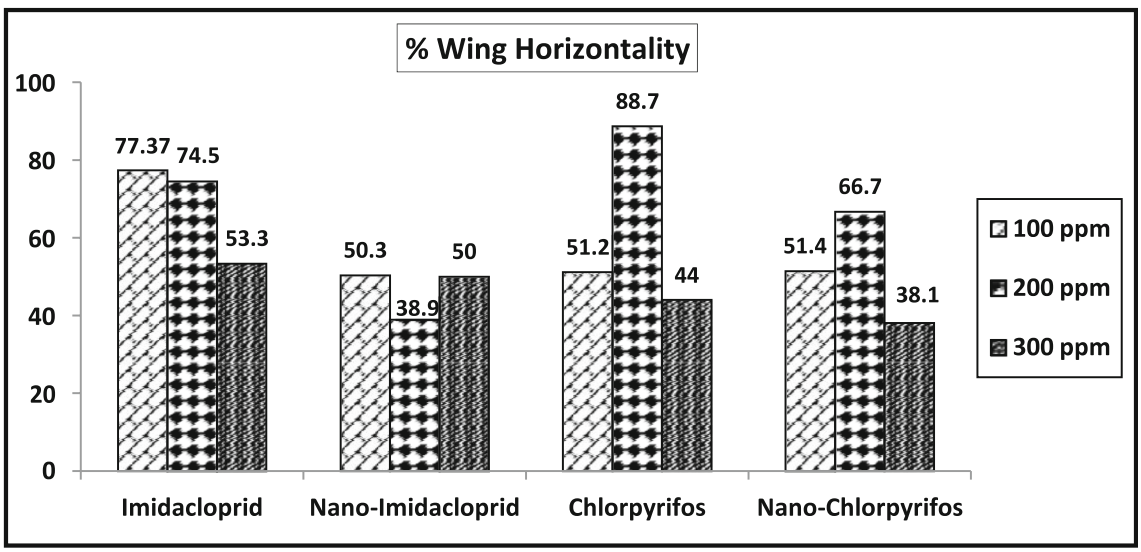

Fig. 3 Effect of three concentrations/insecticide (%) on wing horizontality of honeybee worker

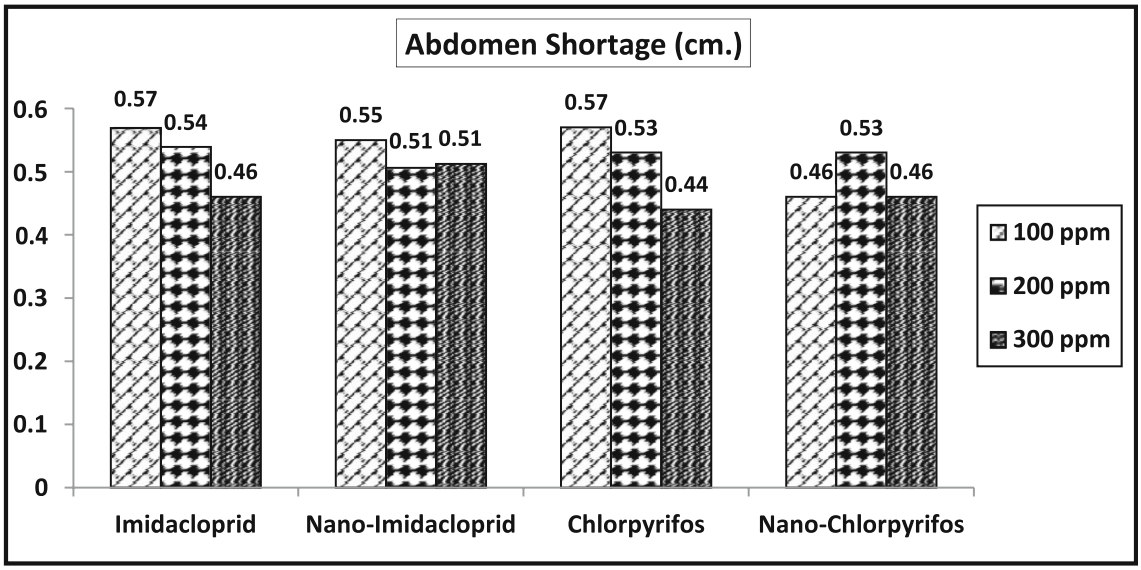

Fig. 4 Effect of three concentrations/insecticide on abdomen shortage of honeybee worker

workers of honeybee "*Apis mellifera* L.", they suggested that the latter showed a 48-h oral LD50 value (153 ng/ bee), about five folds higher than that of imidacloprid (30 ng/bee). Also, they stated that summer bees was higher sensitively than winter bees because the lowest observed effect concentration (LOEC) of imidacloprid was higher in winter bees (48 μg/kg^{-1}) than in summer bees (12 μg/kg^{-1}). Moreover, Suchail et al. (2004) suggested the half – life of imidacloprid for honeybee reached to 5 h.

Cresswell et al. (2012) stated that honeybee have an adaptation to feed on nectars containing synthetic alkaloids, such as imidacloprid than bumble bees because they are feeding on tropical nectars which contain natural alkaloids. Nevertheless, sublethal effects such as weakness of movements and feeding failure happened. On the same trend, Williamson et al. (2014) conducted that honeybee suffered from a shortage of certain major functions like inability to right themselves after down and notice ably sluggishness depended on dose and concentration.

It is worth mentioning that these previous results are inconsistent with studies of (Schmuck et al. 2001; Ramirez-Romero et al. 2005; Yang et al. 2008) whose stated that imidacloprid concentrations in syrup above 20 ng g^{-1} caused decrease foraging behavior of honeybee, while that concentrations above 50 ng g^{-1} convert for aging behavior. Moreover, Brandt et al. (2016) suggested that neonicotinoids make negative effects on immunology characteristics of honeybee and lead to decline of disease resistance. Also, direct lethal effects of neonicotinoids occurred rarely because forager bees exposure to it accidentally (Pistorius et al. 2009). On the other side, most common, lower concentrations of neonicotinoids leading to sublethal effects especially on honeybee movements (Decourtye et al. 2004; Henry et al. 2012).

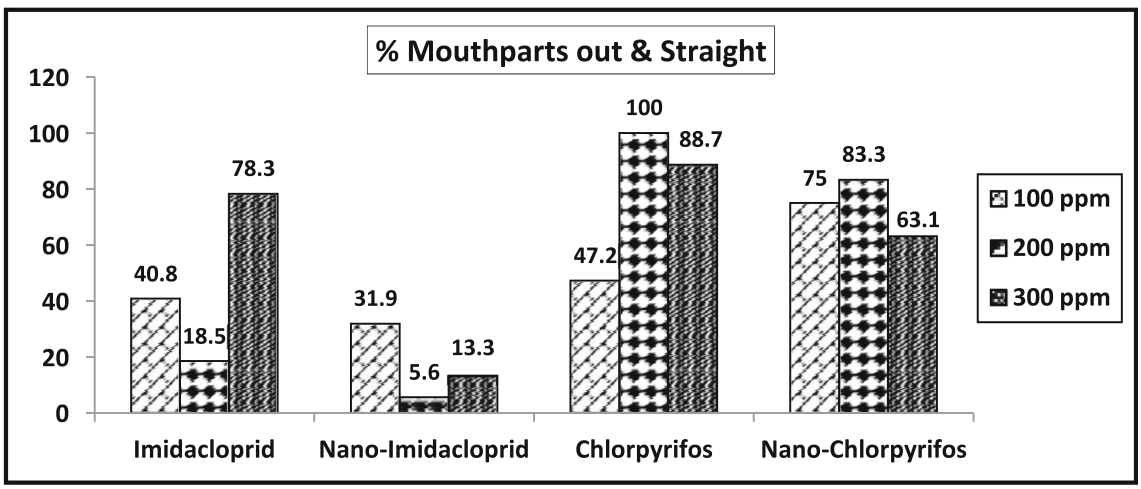

Fig. 5 Effect of three concentrations/insecticide on mouthparts out & straight of honeybee worker

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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