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Lethal and sublethal effects of imidacloprid on development and reproduction of biocontrol agents *Cocinella transversalis* Fabricius (Coleoptera: Coccinellidae) and *Cheilomenes sexmaculata* (Fabricius) (Coleoptera: Coccinellidae)

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Abstract

Transverse and zigzag ladybird beetles, *Coccinella transversalis* Fabricius and *Cheilomenes sexmaculata* (Fabricius) are dominant aphidophagous predators in agricultural ecosystems. The imidacloprid is an insecticide that belongs to the neonicotinoid group which is used for controlling aphids and other sap feeding insect pests, however, it is found to have certain harmful effects on beneficial arthropods. Hence, there is need to analyse the adverse effects of insecticides on beneficial insects to further improve IPM strategies against crop pests. Therefore, the lethal and sublethal effects of imidacloprid on predatory *C. transversalis* and *C. sexmaculata* were tested. The study revealed that the LC_{50} values of imidacloprid against *C. transversalis* and *C. sexmaculata* were 0.02 and 0.06% respectively, which were greater than recommended concentration indicating the less susceptibility of the coccinellids to the insecticide. Residual contact tests were conducted at sublethal doses of insecticide to evaluate the side-effects of these predators starting from the third instars but covering the entire life cycle. Results demonstrated that imidacloprid did not have any significant effects on the developmental time of third instars and adult pre-oviposition period. However there was significant reduction in mating period, oviposition period, fecundity and adult longevity whereas there was significant prolongation of egg incubation period, fourth instar duration and pupal period which ultimately resulted in delay of life cycle when the *C. transversalis* and *C. sexmaculata* were exposed at lethal and sublethal doses of imidacloprid. Thus, the study unfolded that imidacloprid at lethal and sublethal concentrations has the potential to affect predatory coccinellids adversely.

Keywords: Lethal and sublethal effects, *Coccinella transversalis*, *Cheilomenes sexmaculata*, imidacloprid

1. Introduction

The impact of synthetic insecticides on the environment, beneficial insects and human health due to exposure are gaining utmost concern in recent days. Hence it is important to adopt biological control. However bio-control alone may not be effective enough to manage insect pest populations, and sometimes use of selective insecticide treatments is required (Garzon *et al*., 2015) [1]. Imidacloprid is a widely used selective pesticide throughout the world to control aphid infestations in different crops (James, 2003; Devee and Baruah, 2012; Radha, 2013) [2, 3, 4]. At the same time, the activity of coccinellids is much higher in such fields where they feed on aphids and survive.

Generally, acute toxicity of a pesticide on beneficial arthropods is known as a lethal dose (Rahmani and Badani 2016) [5]. However, many researchers have evaluated the acute toxicity of insecticides (median lethal doses or concentrations) on bio-agents and ignore indirect outcomes such as sublethal effects of pesticides on the insect physiology and behavior (Desneux, 2007; Rahmani and Badani, 2013) [6, 7]. It is possible to understand the overall toxicity of pesticides on beneficial organisms more accurately with help of life table analysis (Kim *et al.,* 2004) [8]. Lethal and sublethal effects of many insecticides on crop pests and their predators have been evaluated (Jiang *et al*., 2018; Khan *et al.,* 2018) [9, 10]. However, studies regarding the potential lethal and sublethal effects of imidacloprid on the ladybird beetles

Coccinella transversalis and *Cheilomenes sexmaculata* were not widely reported (Papachristos and Milonas, 2008; Aswathi *et al.*, 2013; Yu *et al.*, 2014) [11-13]. The purpose of the present study was to explore the potential effects of imidacloprid at lethal (LC_{50}) and sublethal concentrations (LC_{30}) on developmental and reproductive parameters of predatory coccinellid beetles. The third instars of *C. transversalis* and *C. sexmaculata* were tested in the laboratory to determine the effects on development and reproduction. The result provides an available reference for optimizing the use of imidacloprid as effective components of Integrated Pest Management (IPM) strategies in agriculture.

2. Materials and methods

2.1. Mass culturing of the prey *Aphis craccivora* Koch. (Aphididae: Hemiptera) and their predators *Coccinella transversalis* and *Cheilomenes sexmaculata*

Aphis craccivora (collected from ICR farm, AAU, Jorhat) were reared on potted cowpea plants var. Kashi Kanchan. The culture of *A. craccivora* was kept separately in three wooden net cages (1.5×1.5×2 meters) in an air conditioned insectary maintained at 25±2 ºC, 70±5% RH under natural lighting. The commonly observed predatory coccinellids *C. transversalis* and *C. sexmaculata* were collected from ICR farm, AAU, Jorhat. They were reared on *A. craccivora* in cowpea plants. To avoid cannibalism, only ten pairs of coccinellids were released in one chamber and required instars for experiment were collected from different chambers.

2.2. Evaluation LC_{50}, LC_{30} doses of imidacloprid against *Coccinella transversalis* and *Cheilomenes sexmaculata*

For the determination of LC_{50} and LC_{30} values, stock solutions of known strength of the insecticide (10%, 20% and 50% strength by dissolving 10 mg, 20mg and 50mg of insecticide in 100 ml acetone) was prepare from the marketed product imidacloprid 17.8 SL (Confidor). Subsequent concentrations (0.01%, 0.02%, 0.05% 0.001%, 0.002% and 0.005%) were prepared from the stock solutions. Insecticide was applied in the form of a dry film, deposited on the inner surface of the Petri dish by following the method Gupta and Rawlin, (1966) [14]. Thin and uniform film was prepared by taking 1 ml of insecticide solution in a Petri dish and rotated till dryness. Toxicity of these films was determined against third instars of *C. transversalis* and *C. sexmaculata* (Jiang *et al*., 2018) [9]. One larva was released into each Petri dish, which served as one replication. Ten replications of each concentration of the insecticide were maintained. Simultaneously, a control set was also run, which had only dry film of acetone. The Petri dishes were kept in an incubator at 28±2 ºC for six hours to expose the insecticide. Then the grubs were transferred to fresh Petri dish without insecticide, provided with cowpea aphids. Petri dishes were covered with a piece of muslin cloth held in position with the help of rubber bands. Petri dishes were kept in incubator at 28±2 ºC and after 24 hours mortality counts were made. Percent larval mortality in each treatment was determined. If mortality occurred in the controls, the observed mortality was corrected by using Abbott's formula (1925) [15]. The dosage mortality data were subjected to probit analysis (Finney, 1952) [16] to find out LC_{50} and LC_{30} values and the regression equation.

2.3. Evaluation of the lethal and sublethal effects on developmental and reproductive parameters of *Coccinella transversalis* and *Cheilomenes sexmaculata*

Third instars were collected from rearing cages and exposed to LC_{50} and LC_{30} doses of imidacloprid in Petri dish (90 mm dia.) for 6 hours (Jiang *et al.*, 2018) [9]. The grubs were transferred to other Petri dishes (without insecticide residues) individually to avoid cannibalism and provided *A. craccivora* as food. Ten individuals of coccinellids were taken as one treatment. Third instars (L_3) were chosen for exposure to insecticide because their natural mortality is less in comparison to first and second instars (Rahmani and Badani, 2016) [5]. If mortality was observed in control it would be corrected by using Abbott's formula (1925) [15].

2.4. Developmental parameters of *Coccinella transversalis* and *Cheilomenes sexmaculata*

The parameters including third instar (L_3) period, fourth instar (L_4) period, pupal period and adult longevity were recorded. Subsequent molts were determined by the presence of exuviae.

2.5. Reproductive parameters of *Coccinella transversalis* and *Cheilomenes sexmaculata*

To observe the reproductive parameters, three pairs of freshly emerged beetles (male and female) were placed in a beaker (20×8 cm) covered with muslin cloth. The sexes were differentiated by observing the size of abdomen, pigmentation on elytra and other morphological characters. The adults were provided *A. craccivora* with leaves and observed daily at regular intervals for recording the mating period. After completion of mating, the males and females were separated, supplied with food and observed for egg deposition. The pre-oviposition period, oviposition period, fecundity (number of eggs laid by a female individual) and adult longevity (duration from adult emergence to death of individual) were observed. The incubation period was recorded from the time of oviposition to date of hatching.

3. Statistical analysis

The LC_{50} and LC_{30} (the concentration causing 50% and 30% of the test species to die) were determined by a log-probit regression analysis. The lethal and sublethal effects of imidacloprid on life stages of *C. transversalis* and *C. sexmaculata* were assessed by one way analysis of variance, and the means were grouped using Tukey's honest significant difference (HSD) tests ($P<0.05$). Statistical analysis was carried out using the SPSS statistical software package version 20.

4. Results

Concentration-response bioassay showed that values of LC_{50} for the third instars of *C. transversalis* and *C. sexmaculata* were 0.02 and 0.06% respectively (Table1). The concentrations that caused 30% mortality of *C. transversalis* and *C. sexmaculata* were 0.006 and 0.002% respectively (Table 2).

4.1. Effects of imidacloprid on *Coccinella transversalis*

Imidacloprid did not have any significant effect on the developmental time of the third instars (F=1.77, P=0.19) and adult pre-oviposition period (F=0.23, P=0.87) (Table 3) but it significantly affected the developmental time of fourth instars (F=5.60, P=0.008), pupae (F=7.17, P=0.002), mating period (F=5.79, P=0.007), oviposition period (F=15.18, P=0.0001), egg incubation period (F=7.61, P=0.002), fecundity (F=15.70, P=0.0001) and adult longevity (F=8.31, P=0.001). The developmental time of fourth instar was significantly shortened in control and LC_{30} *viz.* 3.20 days and 3.80 days respectively, while it is longer in LC_{50} (4.40 days) treatment (Table 3). Also, the pupal developmental time was affected by the insecticide lethal and sublethal treatments i.e. pupal developmental time in the controls was 2.80 days whilst it was 3.60 days in LC_{30} treatment and 4.20 days in LC_{50} treatment which differed significantly from the control. The mating period of 0.80 days in the LC_{50} treatment was significantly shorter as compared to LC_{30} and control treatments *viz.* 1.70 and 2.00 days respectively. Similarly, fecundity of adult females was significantly reduced when treated with LC_{50} dose 108.00 as compared to LC_{30} dose 300.00 eggs and control of 314.00 eggs per female. In addition, oviposition period was significantly different for all treatments while the egg incubation period and adult longevity was significantly longer in controls compared to the LC_{50} treatment ($P<0.5$).

4.2. Effects of imidacloprid on *C. sexmaculata*

Imidacloprid did not have any significant effect on the developmental time of the third instars (F=0.78, P=0.25) (Table 4) but it significantly affected the developmental time of fourth instars (F=6.16, P=0.01), pupae (F=5.16, P=0.02), mating period (F=17.20, P=0.0003), adult pre-oviposition period (F=4.31, P=0.02), oviposition period (F=17.28, P=0.0003), egg incubation period (F=7.40, P=0.008), fecundity (F=12.00, P=0.001) and adult longevity (F=52.67, P=0.0001). Fourth instar development, pupa development and egg incubation period were significantly shorter in control treatments that in LC_{50} treatments (Table 4). The mating period of 1.00 days in LC_{50} and 1.20 days in LC_{30} treatments were significantly shorter as compared 2.40 days in the case of control treatment. Fecundity of females was significantly reduced when treated with LC_{50} dose as compared to LC_{30} dose and control. In addition, oviposition period and adult longevity was significantly different for all treatments with the days shortening as the concentration increased.

Table 1: LC_{50} values of imidacloprid against third instars of *C. transversalis* and *C. sexmaculata.*

insecticide	*Coccinella transversalis*						*Cheilomenessexmaculata*					
	Regression equation (24 hrs)	Df	Standard error of regression coefficient	Heterogeneity (X^2)	LC_{50} (%)	Fiducial limit	Regression equation (24 hrs)	Df	Standard error of regression coefficient	Heterogenity (X^2)	LC_{50} (%)	Fiducial limit
Imidacloprid	Y=1.46+ 0.89X	28	0.053	157.340	0.020	0.017 0.037	Y=0.32+ 0.39X	28	0.039	219.020	0.060	0.190 7.450

X^2 is significant ($p<0.05$).

Table 2: LC_{30} values of imidacloprid against third instars of *C. transversali* sand *C. sexmaculata*

Insecticide	*Coccinella transversalis*						*Cheilomenessexmaculata*					
	Regression equation (24 hrs)	Df	Standard error of regression coefficient	Heterogeneity (X^2)	LC_{30} (%)	Fiducial limit	Regression equation (24 hrs)	Df	Standard error of regression coefficient	Heterogenity (X^2)	LC_{30} (%)	Fiducial limit
Imidacloprid	Y=1.46+ 0.89X	28	0.053	157.340	0.006	0.004 0.008	Y=0.32+ 0.39X	28	0.039	219.020	0.002	0.001 0.004

X^2 is significant ($p<0.05$)

Table 3: Effects on development and reproduction in *Coccinella transversalis* after the third instars treated by lethal and sublethal concentrations of imidacloprid

Parameters	Third instar development (days)	Fourth instar development (days)	Pupa development (days)	Mating period (days)	Adult pre-oviposition period (days)	Oviposition period (days)	Egg incubation period (days)	Fecundity (eggs/ female)	Adult all Longevity (days)
Control	1.40^a	3.20^b	2.80^b	2.00^a	4.00^a	27.80^a	2.70^b	314.00^a	36.40^a
LC_{30}	1.60^a	3.80^b	3.60^{ab}	1.70^a	4.40^a	17.20^b	3.50^{ab}	300.00^a	26.40^{ab}
LC_{50}	2.00^a	4.40^a	4.20^a	0.80^b	5.00^a	8.60^c	4.10^a	108.00^b	14.80^b
P	0.19	0.008	0.002	0.007	0.87	0.0001	0.002	0.0001	0.001
F	1.77	5.60	7.17	5.79	0.23	15.18	7.61	15.70	8.31
Df	3,16	3,16	3,16	3,16	3,16	3,16	3,16	3,16	3,16

Means in a column followed by different letters are significantly different ($P<0.05$).

Table 4: Effects on development and reproduction in *Cheilomenes sexmaculata*the third instars treated by lethal and sublethal concentrations of imidacloprid

Parameters	Third instar development (days)	Fourth instar development (days)	Pupa development (days)	Mating period (days)	Adult pre-oviposition period (days)	Oviposition period (days)	Egg incubation period (days)	Fecundity (eggs/female)	Adult all Longevity (days)
Control	2.20^a	2.80^b	4.20^b	2.40^a	4.20^a	19.20^a	2.70^b	266.00^a	79.60^a
LC_{30}	2.40^a	3.60^{ab}	5.20^{ab}	1.20^b	5.20^a	15.00^b	3.20^{ab}	260.00^a	53.20^b
LC_{50}	2.40^a	4.20^a	5.40^a	1.00^b	5.40^a	11.20^c	4.30^a	184.00^b	40.60^c
P	0.78	0.01	0.02	0.0003	0.02	0.0003	0.008	0.001	0.0001
F	0.25	6.16	5.16	17.20	4.31	17.28	7.40	12.00	52.67
Df	2,12	2,12	2,12	2,12	2,12	2,12	2,12	2,12	2,12

Means in a column followed by different letters are significantly different ($P<0.05$).

5. Discussion

The investigation was conducted for the first time to study lethal and sublethal effects of imidacloprid, a neonicotinoid insecticide, on two predatory lady beetles (*C. transversalis* and *C. sexmaculata*). Other insecticides belonging to the neonicotinoid group including thiamethoxam, acetamiprid, clothianidin and dinotefuran, are commonly used in greenhouses and/or in the other crops in order to control various phytophagous insects such as aphids, whiteflies and mealybugs where its natural enemies such as lady beetles are active (James, 2003; Cloyd and Bethke, 2011) [2, 17]. Beneficial insects were exposed to these insecticides through direct interception, by the ingestion of pesticide-contaminated prey, or by indirect contact with insecticide residues on surface of plants (Jepson, 1989) [18].

The results from our study demonstrated that LC_{50} values of imidacloprid on third instars of *C. transversalis* and *C. sexmaculata* were 0.02 and 0.06% respectively, which were greater than the manufactures (Bayer) recommended concentration. This toxicity data shows that *C. transversalis* and *C. sexmaculata* were not very susceptible to this insecticide and it is very unlikely to be exposed to such a concentration (LC_{50}) in the field. Our data is supported by data obtained by Bozsik (2006) [19], who concluded that the adults of *C. septempunctata* are not as susceptible to imidacloprid, because the LC_{50} of the insecticide on the beetle was more than the recommended concentration. Similarly, Jalali *et al*. (2009) [20] also identified that the adult *Adalia bipunctata* were less susceptible to imidacloprid. In contrast James (2003) [2] reported that imidacloprid was highly toxic to the lady beetle *Harmonia axyrids*, and it produced 80% mortality at the field recommended concentration.

Though it is demonstrated that both the predatory coccinellid species are less susceptible to imidacloprid the long term exposure to lethal and sublethal concentrations adversely affected the developmental and reproductive parameters under laboratory conditions. The experiment started with third instars (exposed by contact with residual film) which allowed the study of delayed treatment-related effects on all life-stages. The developmental time of fourth instars, pupae, mating period, oviposition period, egg incubation period, fecundity and adult longevity were adversely affected. However, the developmental time of third instar and adult pre-oviposition period was not affected significantly. In another study imidacloprid shortened the adult life span of the predator by 25 and 32 days in females and males, respectively, extended pre-oviposition period and decreased fecundity in *Hippodamia undecimnotata* (Papachristos and Milonas, 2008) [11]. Khani *et al*. (2012) [21] demonstrated that an imidacloprid sublethal concentration has adverse effect on the ladybird *Cryptolaemus montrouzieri* Mulsant. However, Araya *et al*. (2010) [22] observed that imidacloprid has no harmful effect on other beneficial arthropods such as *Chrysoperla carnea* (Stephens) and *Aphidius ervi* (Haliday). Mahdian *et al*. (2007) [23] also found no adverse effects with sublethal doses of imidacloprid on the predatory stinkbug *Pycromerus bidens*. The susceptibility of different organisms is varied due to differences in target-site sensitivity, quality and quantity of enzyme activities of the individual organisms (Cho *et al*., 2002) [24]. The results achieved in this study found that imidacloprid at both lethal and sublethal concentrations has potential to adversely affect the predatory ladybirds *C. transversalis* and *C. sexmaculata* which was also strengthens the findings of Smith and Krischik (1999) [25], Elzen (2001) [26] and Yu *et al*. (2014) [13].

6. Conclusion

The current data indicates that imidacloprid has potential risks for beneficial non-target coccinellids by directly affecting life history parameters. However, regarding the long-term insecticidal efficiency of imidacloprid to aphids and other soft-bodied insects, it can be indirectly detrimental by removing the food from the predators. The study may also suggest avoid spraying imidacloprid on crops wherever coccinellid beetles are actively present.

8. References

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