Article

A review of imidacloprid toxicity in coccinellids

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Abstract

Insecticides are the pesticides that are used to deter pests as they affect their nervous system, respiration, growth and by harming their exoskeletons. Meanwhile, they are affecting the non-target natural enemies such as ladybirds that are frequently used in IPM as biological control agents. Insecticides were presumed to be ineffective for Coccinellids earlier, but research studies have shown that these insecticides severely effect physiological and behavioural patterns of natural predators leading them to death. A best-selling insecticide, Imidacloprid: a neurotoxin belonging to neonicotinoid affects the behaviour and performance of natural enemies by effecting their fecundity, egg hatching, developmental time, growth rate, locomotion, survival rate and causing mortality of various Coccinellids including: *Hippodamia undecimnotata*, *Coccinella septempunctata*, *Harmonia axyridis*, *Coleomegilla maculata*, *Hippodamia convergens*, *Serangium japonicum*, *Hippodamia variegata*, *Coccinella novemnotata.*

Keywords insecticides; imidacloprid; neurotoxin; coccinellids; mortality; fecundity.

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1 Introduction

Pests belong to a broad spectrum of organisms including insects, whiteflies, aphids, mites, ticks (and other arthropods). There are many chemicals including insecticides, insecticidal soaps, horticultural oils, azadiractin that have been very effective for controlling pests like aphids and greenhouse whiteflies (Miller and Uetz, 1998).

Insecticides are known pesticides that vary in their activity i.e., disturb the nervous system, harm their exoskeletons are used to execute or deter pests (NPIC). Since the introduction of several synthetics insecticides including organophosphates (OP; 1960s; Zhang et al., 2011; Jafarbeigi et al., 2014; Zhang, 2018), carbamates (1970s), pyrethroids (1980s) and neonicotinoids (late 1980s) an increase in agricultural output has been observed due to their ability to control pests (Akhtar et al., 2009). Insecticides may be neurotoxins (imidacloprid and Lambda-cyhalothrin), respiration inhibitors (organotins and propargite), growth inhibitors and regulators (Hexaflumuron and Pyriproxyfen) and stomach poisons (deltamethrin). Their effectiveness is usually assessed by their dose which are median effective concentration (LC50) or also called median lethal concentration (LC50) and lethal median dose (LD_{50}) to substitute the different species of arthropods (Sánchez-Bayo, 2011).

1.1 Chemical control in IPM (Integrated Pest Management)

To control aphids, the development of integrated control system has gained much importance in most of the parts of the world. While insecticides are promoted throughout the world, assumed to be well-suited to natural predators however, data documenting their effects on the biological control agents is available (Smith and Krischik, 2000). Many species of ladybirds have been frequently used in IPM and biological control programmes because their different life stages can show diverse responses to the same pesticide (Skouras et al., 2017). For the biological control of pests including aphids, the use of ladybird beetles was noteworthy (Seo and Youn, 2002).

1.2 Family coccinellidae

The members of family coccinellidae are often termed as Ladybirds or Ladybugs and the word coccinellid is derivative of a Latin word *coccineus* which means "scarlet" (Mashori et al., 2016). The Coccinellids are familiar due to their unique striking appearance and convex body shape. They are small, not longer than 4-9 mm, and can be small up to 1 mm (Koren et al., 2012). They have a rounded body but sometimes it may be may be elongated, oval and flattened. Their body color varies, mostly they are reddish, black, orange and straw colored. Coccinellids live in all terrestrial ecosystems such as forests, grasslands, agro ecosystems, plains and mountains (Zahoori et al., 2003)

Owing to be predacious in nature, approximately 90% of coccinellid species are propitious and they prey upon homopteran pests of citrus including scales, whiteflies, aphids and psyllid and play important role in controlling pests, especially aphids, scale insects, mites and soft bodied insects (Slipinski, 2007). Coccinellid beetles are negatively geotactic and positively phototactic, therefore, they are often found on the apex of plants (Wiles and Jepson, 1994) where there is greatest amount of pesticide residues (Cilgi and Jepson, 1992). This may result in variable susceptivity of coccinellids to insecticide residues. *Coccinella septempunctata* L. is the most common species of ladybird beetle family coccinellidae and it preys on more than 20 aphid species (Skouras et al., 2017).

1.3 Need for insecticides

Horne and Page (2008) stated that the performance of these insects has not been so effective in the fields in many cases and the principle solution for controlling pests remains in the use of insecticides. Now a day, selective insecticides are being used for this purpose and are of known importance for the conservation of coccinellids in Integrated Pest Management Programs (Skouras et al., 2017).

1.4 Neonicotinoids

Neonicotinoids is the most important and popular class of insecticides introduced to the global now a day and are registered in more than 120 countries (Tan et al., 2012). According to a report in 2008, Global insecticides market comprises 24% neonicotinoids (Jeschke et al., 2011). Neonicotinoids mostly acts as neurotoxins for different arthropods and are and are amongst the most effective insecticides for the control of pests i.e., aphids, whiteflies, leaf and planthoppers world-wide (Goulson, 2013).

They bind irreversibly to the Nicotinic Acetylcholine Receptors on the post synaptic membrane, disrupting neural transmission and overstimulate the nerve cells, paralysing the insect (Tomizawa and Casida, 2003 & 2005; Simon-Delso et al., 2015). Neurotoxic insecticides may have adverse effects on the behavior of natural enemies (Haynes, 1998). Negative impacts of insecticides on natural enemies can be reduced by making a good choice of insecticide, dosage, or insecticide application timing (Obrycki and Kring, 1998).

1.5 Effects of insecticides on non-targeted insects

Preferably, insecticides ought to be lethal only for the targeted pests contradictally, they have affected different non-targeted insects in different ways.The non-targeted arthropods including coccinellids are badly affected when they forage in the crops treated with neonicotinoid insecticides, or prey on the organisms infected by the efficacious elements of different insecticides (He et al., 2012). They have an impact on survival, growth, development, reproduction and behavior of natural predators thereby, affecting their physiological and biochemical processes causing mortality (Desneux et al., 2007; Fogelet al., 2013).

1.6 Effects of neonicotinoids insecticides on coccinellidae predators

The neonicotinoid insecticides are neurotoxic insecticides that effect the behaviour and performance of natural enemies i.e. coccinellids, negatively by causing impairment in their physiological and behavioural traits (Cabral et al., 2011; Fogel et al., 2013; He et al., 2012) and may cause changes to fecundity, longevity and egg viability (Ruberson et al., 1998), foraging patterns alterations, disturb sexual communication and host recognition (Elzen, 1989) and the velocity with which an insect walks. The amount of insecticide transferred from plant to insect while walking and the area of the leaves they contact with, influence mortality levels.

1.7 Imidacloprid: A neurotoxin

Imidacloprid, 1-(6-chloro-3- pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine, was the first neonicotinoid appeared in global market in 1991(Jeschkeet al., 2011; Ravikanth et al., 2017). It is the best-selling insecticide used worldwide (Nauen and Bretschneider, 2002). Imidacloprid is classified as moderately dangerous (Class II WHO; toxicity category II EPA). As it is a neurotoxin, it causes loss of co-ordination, tremors and paralysis in insects (Papachristos and Milonas, 2008).

Just as its binding properties are more compatible with insect's Nicotinic Acetylcholine Receptors (nAChRs) Imidacloprid is safe for vertebrates (Tomizawa and Casida, 2003). There are physiological differences between Nicotinic Acetylcholine Receptors (nAChRs) of mammals and insects which increases insect's sensitivity towards Imidacloprid and give mammals a high level of safety (Tomizawa and Casida, 2003).

Imidacloprid can be used in a variety of useful ways such as microcosm, sprays, by treating glass plates, leaf dip method on cotton etc. But when given through soil, it completely degrades and disappears under temperate climate conditions as it is degradable in organic soils (Krohn and Hellpointner, 2002) so, the best way to use imidacloprid is as foliar spray. Imidacloprid has been an effective insecticide in pest systems where pests are resistant to other compounds (Nauen and Denholm, 2005). It is useful against some Coleoptera, Diptera and Lepidoptera (Creary, 2009).

Coccinellids are less sensitive to insecticides than other pests (Al-Doghairi et al., 2004) but their susceptibility varies with different insecticides and there is a need to study the lethal and sub-lethal effects of insecticides on non-target, natural predator arthropods e.g. Coccinellidae predators (Desneux et al., 2007). For assessing the efficacy of insecticides on natural predators, following procedure can be used. Firstly, in laboratory bioassays, insecticidal effects on the biological control agent are determined and if insecticide cause no harmful effects then the tested insecticide is compatible with the biological and physiological processes of natural predators. But if the pesticide is showing harmful effects then there is a need for further testing in semi-field and field situations (Smith and Krischik, 2000).

2 Effect of Imidacloprid on *Hippodamia undecimnotata*

Insecticides has serious effects in the performance of both immature and adult *Hippodamia undecimnotata*. When *H. undecimnotata* prey upon aphids on the plants that has been sprayed on by insecticides, their behaviour is affected (Papachristos et al., 2008) performed experiments and documented the effects of imidacloprid on *H. undecimnotata* when exposed to it. Imidacloprid caused the mortality rate of *H. undecimnotata* to increase and also caused lighter adults to lay fewer eggs and have a shorter life span. Compared to controls, the weight of adult is not affected by imidacloprid and when imidacloprid was applied on soil pre-oviposition period, fecundity and lifespan *H. undecimnotata* also affected.

a) Developmental time

It was observed in the experiment that developmental time of pupa was shorter for the females that were reared on the plants treated with imidacloprid as compared to those on control plant and ranges from 5.4 to 5.9.

b) Survival Rate

Imidacloprid influenced the survival of *H. undecimnotata* larva. The survival rate of immature predator stages was lower because of high mortality of first instar larvae. The survival rate of the adults, reared on the untreated plants was very high in contrast to plants treated with imidacloprid. The percentage survival of immature stages (larva and pupa) of beetles reared in control system and on the plants treated with imidacloprid was 77.4% and 52.2% respectively.

c) Fecundity of the Females

725.3 eggs per female were produced by the females reared on imidacloprid treated plants and 1089 eggs per female were produced by females of the control groups. The maximum production of eggs for control and females treated with imidacloprid was 28 eggs per female per day.

d) Egg Hatching

There was no significant difference in the egg hatchability. However, egg hatchability of *H. undecimnotata* was lower for imidacloprid than those in the control system.

3 Effect of Imidacloprid on *Coccinella septempunctata*

The severe toxicity of different insecticides and their resistance mechanisms has been investigated in *C. septempunctata* but imidacloprid is safe or less toxic for *C. septempunctata* (Bozsik, 2006). However, according to a recent study by Xiao et al. (2016), toxicity of imidacloprid on *C. septempunctata* is not well documented yet. Xiao et al. (2016) found through experiment that the biological performances of *C. septempunctata* were affected badly by the sub-lethal concentrations of imidacloprid. Because of exposure to imidacloprid developmental duration of juvenile extends, fecundity lowers and lifespan shortened.

In the experiments performed to check the toxicity of Imidacloprid, 2nd instar larvae of *C. septempunctata* obtained from laboratory culture were used as test organisms and under different dosses of imidacloprid various effects on the biological and physiological parameters i.e. development period, survival rate, adult emergence, mortality, walk rate and predation rate were observed in 2^{nd} instar larva of *C. septempunctata* (Yu et al., 2014). Long-term effects of a single application of imidacloprid on *C. septempunctata* L. were studied in laboratory microcosms and the test procedure started with the 2nd instar larvae and covering their complete life cycle. The experiment was performed in an enclosure containing pot filled with soil planted with broad bean plants. Through experiment, measurement endpoints i.e. development period, pupation percentage, hatching percentage, adult emergence, mortality and egg production were assessed (Yu et al., 2014). When sprayed with imidacloprid their fecundity was affected 6.99% and longevity was affected by 26.8% (Xiao et al., 2015).

a) Developmental time

Developmental time of female L_1 instars were increased by application of Imidacloprid (2.3 days) compared to control (2.2 days). The susceptibility of different larval instars of *C. septempunctata* is in the following order; 1^{st} Instar Larva > 2^{nd} Instar Larva > 3^{rd} Instar Larva > 4^{th} Instar Larva (Skouras et al., 2017). In laboratory microcosm test, when imidacloprid was applied once on the instar larvae of *C. septempunctata* their survival rate decreased from the 2nd day of application (test period 0–18 days) and in the control systems where larvae obtained imidacloprid in 13.66 and 6.83 g a.i. ha^{-1} their survival rate decreased in 3-18 days after insecticide was applied (Yu et al., 2014).

b) Mortality rate

Skouras et al. (2017) stated that mortality rates of *C. septempunctata* can be increased through residual toxicity and by feeding on aphids treated with imidacloprid. In the microcosm test performed by Yu et al. (2014), mortality of larvae steadily increased in control and average 62.5% survival of larvae was observed at the end of the 18th day experiment. On 18th day, the longevity of the first generation larval stages in the 3.42 g a.i. ha-1 treatment was decreased to average 40%.

c) Weight

Imidacloprid has effect on the weight of adult *C. septempunctata*. Skouras et al. (2017) observed that when treated with imidacloprid (LD_{10}) the weight of the adults was lower than the adults treated with other insecticides.

d) Predation Rate

Imidacloprid also lowered the daily predation rate during the 4th instar larva than any other insecticides (Skouras et al., 2017). The microcosm test values for lowest L (E)R50 and NOERs are lower than that obtained from the application of imidacloprid in the field (Yu et al., 2014). The data obtained from experiments clearly illustrate that potential effects of imidacloprid on C. septempunctata could not be excluded at field application rates higher than 3.42 g a.i. ha^{-1}.

4 Effect of Synthetic Imidacloprid on *Harmonia axyridis*

Harmonia axyridus (Pallas)is commonly known as multicolored Asian ladybird beetle and is common to a wide range of habitats all around the world (Youn et al., 2003). Larvae of *H. axyridis* were killed by spraying imidacloprid directly on plants, also they fell from the plants by knockdown effect when encounter imidacloprid. In adverse conditions, their mortality rate increased (Vincent et al., 2000).

a) Larva Production

When the eggs of *H. axyridis* were exposed to imidacloprid, larva did not produce.

b) Survival Rate

Survival rate was zero when *H. axyridis* were exposed to Imidacloprid.

c) Mortality

Skouras et al. (2017) documented that imidacloprid cause 100% mortality in 1st and 2nd instar larvae of *H. axyridus* at a dose of 50mg a.i./ L. When *H. axyridis* were directly exposed, Imidacloprid caused mortality of eggs and early instars of *H. axyridis*. At recommended concentrations only 25% of the third instars were survive but the eggs, 1st and 2nd instars were not survived. Imidacloprid is highly toxic for early stages of *H. axyridis* (Youn *et al*., 2003).

d) Locomotory behavior

The locomotory behavior of *H. axyridis* larvae was affected when they contact with imidacloprid. Imidacloprid caused an increase in their number of steps (s^{-1}) and angular speed (deg. s^{-1}). When larvae were exposed to imidacloprid treated plates for a long as 5 minutes, changes in their locomotory behavior occur rapidly and proceed to knockdown effect (showed a state of intoxication and partial paralysis proceeds to death). Larvae recovered from their knock down state but the changes in their locomotory behavior lasts 24 hours after their contact with plates treated with imidacloprid.

5 Effect of Imidacloprid on *Coleomegilla maculata*

Coleomegilla maculata is selected as a test organism for two reasons; it is a facultative pollen feeder and can complete their life cycle on only pollens also it is an agent for biological control (Hodek and Honek, 1997). In experiments, the effects of imidacloprid on days to first oviposition, survivorship, daily egg production and some of the behavioural parameters including walk rate and flip time of *C. maculata* were observed and measured. *C. maculata* mobility decreases when exposed to imidacloprid (Smith and Krischik, 1999; Krischik et al., 2015).

a) Walk Rate

When beetles were exposed to imidacloprid their walk rate was observed by placing them on a piece of paper (75 g/m^2). When beetle walked on the paper their path was marked with the help of pencil and the time they spend was recorded. A string was used to measure the length of path travelled. An individual beetle spent about 1.31 to 30s and those who did not show any movement during these 30 seconds were given a zero score.

b) Flip time

In addition to walk rate, flip time was also studied to examine the reduction in their mobility due to exposure to the plants treated with imidacloprid. Each beetle was placed on a small piece of Fisher brand medium prosity filter paper with their dorsal side down andtheir flip time (time in which beetle turned itself erect) was observed. It was given a score of 31 if a beetle was not capable to right itself in 30 s. The Beetles that died during first 7 days of the bioassay were not included in the behavioral parameter's study.

After behavioural parameters, the parameters for fitness of beetles were examined by placing them in the petri dishes of size 60 by 50 mm. Their days to 1st oviposition, daily egg production and survival rate was observed for about 30 days.Centrifuge tube (0.5 ml) filled with distilled water (tube was refilled with water when needed.) and Cotton was placed in the petri dish (Smith and Krischik, 1999).

6 Effect of Imidacloprid on *Hippodamia convergens*

Imidacloprid has lethal effects on *Hippodamia convergens* adultswhen applied topically. Imidacloprid-treated plates didn’t affect larval mortality and has no effect on larval growth. However, changes in weight gain per dayoccur. Larvae who contacted the imidacloprid residue showed a state of intoxication and partial paralysis proceeds to death (Vincent et al., 2000). When several species of coccinellids including *Hippodamia convergens* were exposed to imidacloprid residues significant mortality rate was observed (Mizell and Sconyers, 1992).

7 Effects of Imidacloprid on *Serangium japonicum*

The effects of insecticides on Coccinellids are rarely documented in the past (Wang and Shen, 2002; Papachristos and Milonas, 2008) also there is no information available on sub-lethal effects of insecticides on *S. japonicum.* Imidacloprid reduced predation rate of *S. japonicum* up to 41%. The effects did not stay longer; predators recover rapidly, and the sub-lethal effects disappeared within few hours of exposure to imidacloprid. When sprayed on *B. tabaci* eggs imidacloprid is toxic but is not toxic for *S. japonicum* when they consume *B. tabaci* eggs (He et al., 2012). However, systemically applied imidacloprid neither showed toxic effects on *S. japonicum* nor the functional responses of the predator were affected however, their development and reproductive capacity was affected which resulted in reduced *population growth of S. japonicum.*

8 Effect of Imidacloprid on *Coccinella transversalis*

The impacts of imidacloprid insecticide on the transverse ladybird, *Coccinella transversalis* was tested in acute and long-term toxicity bioassays. In acute toxicity bioassays mortality rate of *C. transversalis* was measured after 72 h of their exposure to wet sprays and dry residues in contrast, mortality however, sublethal effects over a generation was measured in long-term bioassays. The results of acute toxicity tests showed that imidacloprid is lethal and caused complete mortality to *C. transversalis* (Cole et al., 2010).

9 Effect of Imidacloprid on *Hippodomia variegata*

Almasi and Sabahi (2012) documented the toxicity of imidacloprid 350 SC on *Hippodomia variegate* was observed. After 24 h contact, the mortality of adult predator, with imidacloprid was 63.4% and the effect decreased up to 56.1 after 5 days.

10 Effect of Imidacloprid on *Coccinella novemnotata*

There is no much information available on susceptibility of *Coccinella novemnotata* to the insecticides i.e. Imidacloprid (Abdel-Latif and Abdu-Allah, 2010).

11 Conclusion

A lot of works have been done to assess the toxicity of imidacloprid and other insecticides on different not targeted species. As imidacloprid is used world-widely at a large scale there is need of more study to gain knowledge about its effects on beneficial species of coccinellids and other beneficial natural predators. If it is more harmful then measures to reduce the application of insecticides should be taken otherwise potential risks for beneficial non-target Coccinellids will occur in near future.

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