Hormetic effects of neonicotinoid insecticides on *Rhizoglyphus robini* (Acari: Acaridae)

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Highlights:

- Exposure to imidacloprid, thiamethoxam, and dinotefuran, affected the fecundity in *R. robini*
- The highest fecundity in *R. robini* females was observed in matings where only the female was treated with imidacloprid
- Imidacloprid treatment increased the activity of detoxifying enzymes in females of *R. robini*.
- Diazinon toxicity was seriously affected after exposure to a hormetic dose of imidacloprid

Abstract

The stimulation of biological processes by sublethal doses of insecticides or other stressors is known as hormesis. Here, we have evaluated whether exposure to field-relevant or low concentrations of neonicotinoids induce changes in the reproductive capacity of the bulb mite *Rhizoglyphus robini* (Acari: Acaridae). Among the tested neonicotinoids imidacloprid, thiamethoxam, and dinotefuran, the highest hormetic effect on the reproduction of *R. robini* occurred 24 h after the 48 h exposure period to imidacloprid at concentrations of 70 and 140 mg a.i./L. Despite the stimulating effects of imidacloprid on mite reproduction, no significant differences were observed in the offspring (F_1) for biological aspects including egg hatch rate, embryonic period and sex ratio, while an increase was found in the duration of development time from egg to adult. Evaluation of the detoxification enzyme activities of treated adults showed that the highest activity of carboxyl/cholinesterases, cytochrome P450s, and glutathione S-transferases was obtained when exposed to 70, 140 and 70 mg a.i./L imidacloprid, immediately after the exposure period, respectively. Also, an increase in the activity of the antioxidant enzyme catalase was observed compared to that of the control. After imidacloprid pretreatment (140 mg a.i./L), the tolerance of adult mites to diazinon was increased about two-fold. This study shows that exposure to imidacloprid can induce hormetic effects on *R. robini* and could severely complicate its control due to a higher reproduction, enhanced detoxification enzyme activities, and increased tolerance against other pesticides.

Keywords: Bulb mite, Hormesis, Imidacloprid, Enzyme induction, Sublethal effects

1. Introduction

Effective control of insect and mite pest populations is achieved in many cases by applying chemical insecticides and acaricides. All arthropods inhabiting the insecticide-treated environment can be exposed to the field applied doses of an insecticide. Over time, the concentration of insecticides in the field decreases due to the progressive degradation and eventually, the insects are exposed to sublethal doses. Exposure of insects to field-relevant or low doses of insecticides can stimulate specific innate biological processes (Cutler and Guedes, 2017; Malbert-Colas et al., 2020). Investigating the sublethal effects of pesticides have recently been explored more thoroughly in insect toxicology and pest management (Desneux et al., 2007).

The biphasic dose-response, characterized by high-dose inhibition and low-dose stimulation by toxic compounds, is called 'hormesis' (Calabrese and Baldwin, 2003; Calabrese et al., 2010). This phenomenon has been associated with pest resurgence and secondary pest outbreaks (Guedes and Cutler, 2014). Such resurgences could not only lead to increased damage to crops but may cause increased pesticide usage, potentially exacerbating non-target effects and leading to the development of resistance to insecticides and environmental pollution (Cutler, 2013; Guedes et al., 2010). Hormesis has been widely reported in arthropods in recent years. The cases reported as hormesis largely include arthropods such as mites, thrips and planthoppers with a short generation time and high reproductive capacities (Chen et al., 2020; Cohen, 2006; Cutler, 2013; James and Price, 2002; Sial et al., 2018; Wu et al., 2020). Behavioral and physiological modifications that occur in response to insecticide stress can be the result of other strategies that the organism uses to keep homeostasis under stress (Desneux et al., 2007). For instance, exposure to pesticides may additionally modify energy metabolism, disrupt fat accumulation, or delay growth (Adamski et al., 2009; Dingha et al., 2004; Piiroinen et al., 2014; Quinlan and Gatehouse, 1981). Although most responses to stress have negative outcomes, a number of them can be useful and increase fitness. Hormetic and homeostatic modifications modulated by sublethal doses of pesticides can also be the result of environmental conditions experienced by the parents which indirectly affect the fitness of offspring (Bonduriansky and Day, 2009; Mousseau and Fox, 1998). For example, hormesis effects were shown to be trans-generationally transmitted in different aphid species (Wang et al., 2017).

Insects and mites are capable of metabolizing and excreting many toxic compounds such as insecticides to certain degrees with the help of detoxifying and protective enzymes to protect their body (Van Leeuwen and Dermauw, 2016; Feyereisen et al., 2015). The most important types of detoxifying enzymes involved in the metabolism of neonicotinoids are carboxyl/cholinesterases (CCE), cytochrome P450 monooxygenases (P450s), and glutathione-S transferases (GSTs) (Bass et al., 2015). Changes in the activity of these enzymes can alter the toxicity of pesticides on the treated species. Exposure to sublethal doses of insecticides by activating oxidative stress can also affect the growth, development, survival, and fertility of living organisms (Ahmad, 1992; Lopez-Martinez et al., 2008).

Neonicotinoids are a class of synthetic insecticides that are widely used in agriculture to protect major crops against the damage caused by an extraordinarily broad spectrum of phytophagous insects (Jeschke and Nauen, 2008), although their application starts to be more restricted in the EU (Jactel et al., 2019). Like nicotine, neonicotinoids are potent agonists of postsynaptic nicotinic acetylcholine receptors (nAChR) (Bass and Field, 2018). Imidacloprid is the first commercialized neonicotinoid and effective against many sucking and certain chewing insects but reported to be ineffective against plant-feeding mites, except for a few species such as the red-legged earth mite, *Halotydeus destructor* (Umina et al., 2019). It was suggested that amino acid differences underlying structural differences in the nAChR subunits are the main causes of this reduced activity of imidacloprid against mites (Van Leeuwen and Dermauw, 2016). Despite their wide use, an increasing number of studies report adverse effects on non-target organisms (Jactel et al., 2019). Several field and laboratory experiments have revealed that the application of neonicotinoid insecticides can lead to enhanced fecundity of some mite species, including *Tetranychus urticae* (Barati and Hejazi, 2015; James and Price, 2002; Szczepaniec et al., 2011).

Bulb mites of the genus *Rhizoglyphus* attack a range of plants of which most are related to members of the Liliaceae family. This group of mites damages a variety of agricultural products such as onion, garlic, other species of *Allium*, *Lilium*, *Hyacinthus*, and also many vegetables, grains, and ornamental crops in storage,

greenhouses, and farms (Diaz et al., 2000). *R. robini* is a very important pest of the corms of the saffron plant in Iran (Bazoobandi et al., 2020). In this species, two morphologically distinct morphs of fighter and non-fighter males occur (Smallegange et al., 2012; Zeeman et al., 2022). The control of this pest is currently based on soil solarization (Gerson et al., 1981), hot-water treatment of bulbs (Conijn, 1992), and pesticide application. Also, the use of entomopathogenic fungi such as *Metarhizium* spp. is put forward as a potential effective control strategy (Konopická et al., 2021). This soil mite can also be considered a common non-target organism (Carter et al., 2004), which feeds on a wide range of natural foods including plant parts, manure, macerated arthropods, and fungi (Gerson et al., 1985; Wooddy and Fashing, 1993). This study aimed to investigate the sublethal effects of neonicotinoids, especially imidacloprid, on the female fecundity and changes in the activity of detoxifying and protective enzymes in treated *R. robini* mites and also the developmental parameters of their offspring (F_1) (trans-generational effects).

2. Material and methods

2.1 Mite rearing

R. robini was extracted from infested saffron corms collected from Neyshabur, Razavi Khorasan province, Iran, and was identified using taxonomic keys (Fan and Zhang, 2003; Fan and Zhang, 2004; Manson, 1972). Mites were reared in Petri dishes containing wet filter paper and sterilized peanuts, and kept in a closed container in the dark, under laboratory conditions of 25°C with >50% RH. Cultures were biweekly refreshed by transferring one hundred individual mites to a new Petri dish containing water and peanuts (Zindel et al., 2013).

2.2 Insecticides and chemical materials

Bioassays were conducted with the commercial formulations of imidacloprid (Confidor SC 35%, Bayer), thiamethoxam (Actara WG 25%, Syngenta), and dinotefuran (Starkle SG 20%, Sumitomo Chemical). Bovine serum albumin, 3,3',5,5'-tetramethylbenzidine (TMBZ), 1- chloro-2,4-dinitrobenzene (CDNB) and Triton X-100, glucose were purchased from Sigma (USA), and Cytochrome C was purchased from Merck (Germany).

2.3 Toxicity bioassays

The insecticide bioassay method was adapted from toxicological studies on *R. robini* described by Chen (1990) with slight modifications. A disc of filter paper was placed in the bottom of the plastic Petri dish with six cm diameter and 1.5 mL of the chemical solution or distilled water (as control) was added, after which 30 female bulb mites of 1- to 2- day- old were placed on to the filter paper. The mites were exposed to the different concentrations of insecticides (up to 2000 mg a.i./L) for 48 h at 25 °C and > 50% humidity in the dark. Each experiment was repeated at least three times under the same conditions.

2.4 Reproduction bioassays

Because insecticide-triggered hormesis tends to occur following exposure to concentrations around the no observable effects concentration (Calabrese, 2013), *R. robini* reproduction was measured at several sublethal concentrations of three neonicotinoids (i.e. imidacloprid, thiamethoxam, and dinotefuran). For these experiments, groups of newly emerged adult females of *R. robini* were exposed to the field dose (FD), one-second (FD/2), one-fourth (FD/4) and two times of field dose (2FD) concentrations of three neonicotinoids or deionized water (control). After the exposure period of 48 h, based on the ability of the bulb mite to ingest inert cellulose-based filter paper (Cohen and Joseph, 1992; Ruzo et al., 1988), the females were placed individually in separate cells of a 96-well plate containing a wet punched filter paper. The number of eggs laid by females was recorded over the following 4 days (Radwan et al., 2003), considering that after these 4 days, the female oviposition rate was not significantly different between treatments over the egg-laying period of about three weeks (Konior et al., 2001). At least 32 adult females were tested for each insecticide concentration.

2.5 Exposure of R. robini couples to imidacloprid

By placing individual tritonymphs into separate vials, newly emerged (< 48 h old) virgin males and females of *R. robini* were obtained, which were then individually exposed to 70 mg a.i./L imidacloprid for 48 h or to deionized water (control). Next, couples in four different combinations (unexposed couple; exposed female; exposed male; and exposed couple) were allowed to mate for 24 h. Oviposition did not occur during the first 24

h after mating and this time was considered the pre-ovipositional period. From the third day (72 hours after treatment), the number of eggs per female was recorded daily for up to four consecutive days. Eight pairs were provided in each treatment and the experiment was repeated three times.

2.6 Trans-generational experiments

To investigate whether the insecticide-induced hormesis in adults can affect the progeny in the next generation (trans-generational effect), 40 adult female mites were treated for 48 h with sublethal concentrations of imidacloprid as previously described. After treatment, the mites were transferred to an insecticide-free Petri dish containing distilled water and filter paper to lay eggs for five hours. The age-synchronized eggs were kept in the Petri dish to determine the hatch rate, embryonic period, duration from egg hatch to adult emergence, and sex ratio of the F_1 progeny. To calculate the rate of egg hatching and the embryonic period, the eggs were checked daily for six days and the hatched larvae were recorded and removed daily. To determine the duration from egg hatch to adult emergence for each treatment, 20 newly hatched larvae were carefully separated with a thin and soft brush and transferred individually to a well of a cavity microscope slide, containing a piece of wet filter paper and a small piece of peanut, and were covered with a coverslip. Due to the high sensitivity of the larvae to humidity, the test containers were stored in a desiccator in room conditions of 25 °C and >50% humidity in the constant dark. The wells were checked daily and the growth stages were recorded until adults emerged and the duration from egg hatch to adult emergence was calculated. The sex ratio was calculated as the percentage of female offspring. Each experiment was repeated at least three times under the same conditions. Larvae that died during the test were excluded from the analysis (Ako et al., 2004).

Data on fecundity, embryonic period, hatch rate of eggs, duration from egg hatch to adult emergence, and sex ratio were analyzed using the general linear model (GLM) and analysis of variance followed by using Fisher's protected least significant difference (LSD) procedure of SAS (SAS version 9.4). Before analyses, data on the sex ratio and egg hatch rate were arcsine-square root transformed.

2.7 Enzymatic assays

To determine enzyme activities of P450s, CCEs, GSTs, and catalase (CAT), about 50 adult female mites were collected and either homogenized immediately or 48 h after the treatment period with different concentrations of imidacloprid. A P450 assay was conducted according to Brogdon et al. (1997) by measuring heme peroxidase activity, using 3,3',5,5' tetramethylbenzidine (TMBZ) and cytochrome C as substrate and standard, respectively. Briefly, the reaction consisted of 40 µL enzyme source, 125 µL potassium phosphate buffer 0.625 M, pH 7.2, 35 µL H_2O_2 3%, and 300 µL of TMBZ solution (0.01 g of TMBZ in 5 mL methanol plus 15 mL of 0.25 M sodium acetate buffer, pH 5.0. The reactions were incubated at room temperature in the dark for 2 h. The absorbance was measured at 450 nm at the endpoint with a spectrophotometer (Unico, Model UV-2100, USA). Monooxygenase levels were expressed as equivalent units of cytochrome P450 mg^{-1} protein using the standard curve of cytochrome C.

CCEs activity was assessed according to the method described by Van Leeuwen et al. (2006) using α-naphthyl acetate (α-NA) (6.4 mM) as the substrate (diluted in phosphate sodium buffer 0.1 M, pH 7.5). Briefly, after homogenization of mites in sodium phosphate buffer (0.1 M, pH 7.5), containing 0.05% (w/v) Triton X-100 and centrifugation (10,000 × g at 4 °C for 15 min), the supernatants were used as enzyme source in for the assays. 25 µL homogenate was added to 50 µL α-NA, 100 µL Fast Blue RR 0.2%, and 225µL phosphate sodium buffer 0.1 M, pH 7.5. Finally, changes in absorbance were measured by a spectrophotometer (Unico, Model UV-2100, USA) at 450 nm at 30 s intervals for 5min at room temperature (25 ± 2 °C). The values estimated for CCE activity were described as nmol naphthol per min per mg protein.

GST activity was determined using the 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate, according to the method of Habig et al. (1974). Enzyme source (200 µL) was added to 200 µL of CDNB and 200 µL of reduced glutathione (GSH; 10 mM). Absorbance was read at 340 nm every 30 s for 5 min using a Unico 1200 Spectrophotometer (UNICO, Dayton, USA). One unit of GSTs activity was determined using the CDNB extinction coefficient of 9.6 mM^{-1} cm^{-1} and was reported as nmol CDNB conjugated/min/mg protein.

Catalase activity was measured by using hydrogen peroxide as the substrate. After homogenizing the mites and centrifugation at 15000 g for 15 min at 4 °C, 20 µL of enzyme samples were added to 240 µL phosphate buffer (50 mM, pH 7) and 210 µL of fresh prepared 30 mM H_2O_2. A decrease in absorbance due to the decomposition of H_2O_2 was recorded at 240 nm for 3 min using a Unico 1200 Spectrophotometer (UNICO, Dayton, USA). Catalase activity was calculated using an extinction coefficient of 39.4 mM^{-1} cm^{-1} and expressed as µmol of H_2O_2 decomposed per minute per milligram protein (Aebi, 1984; Beers and Sizer, 1952).

The protein concentration was measured by the Bradford method (1976) using bovine serum albumin as a standard. Twenty-five µL of enzyme sample was mixed with 475 µL of Bradford reagent and then incubated for 5 min at room temperature and absorbance was measured at 595 nm using a Unico 1200 Spectrophotometer (UNICO, Dayton, USA).

Each enzyme assay was performed in three independent replicates. Analysis of variance followed by Fisher's protected least significant difference (LSD) means separation assessments, were used to determine significant differences in the level of enzyme activity among treatments (GLM Procedure in SAS/STAT version 9.4).

2.8 Evaluation of diazinon toxicity after pretreatment with a hormetic dose of imidacloprid

After preliminary tests and determination of a range of lethal concentrations of diazinon, newly emerged adult females (<48 h old) were exposed to a hormetic dose of imidacloprid (140 mg a.i./L) or to distilled water (control) for 48 hours. Immediately after pretreatment, mites were transferred to different concentrations of diazinon, and mortality was recorded 48 h after diazinon treatment. The experiment was performed with five concentrations of diazinon and 4 replicates of 30 adult female mites per replicate as described in the toxicity bioassay. LC_{50} values, slopes, and 95% confidence intervals were calculated by probit regression analysis using the software POLO-Plus.

3. Results

3.1 Effects of exposure to neonicotinoids on the fecundity of R. robini

As mortality did not exceed 5% at the highest tested concentrations of imidacloprid, thiamethoxam, and dinotefuran (2000 mg a.i./L), the field dose (FD), one-second (FD/2), one-fourth (FD/4) and two times of field dose (2FD) were used for the evaluation of sub-lethal effects. In the treatment with imidacloprid, a significant hormetic effect on mite fecundity (eggs laid) was observed 24 h after the 48 h exposure period when the adult female mites were treated with the FD (140 mg a.i./L) and FD/2 (70 mg a.i./L). This reproductive stimulation effect was also observed in thiamethoxam-treated mites at 25 and 50 mg a.i./L concentrations 24 h after the exposure period. At 48, 72, and 96 h after thiamethoxam exposure, the highest and lowest cumulative mean number of eggs per female were recorded at 25 and 200 mg a.i./L, respectively. Treatment with dinotefuran had no significant effect on the mite fecundity 24 h after the exposure period, but 48 h after treatment, the highest and lowest cumulative average number of eggs per female were recorded at the dinotefuran concentrations of 150 and 75 mg a.i./L, respectively. (Table 1).

3.2 Effects of exposure to imidacloprid on the fecundity of R. robini couples

In four different mating conditions between males and females treated with a hormetic concentration of imidacloprid (70 mg a.i./L), we observed the highest and lowest rate of reproduction in the couples where only the female and both sexes were exposed, respectively, at the third day after exposure. While, no significant difference was observed between different cross states on the fourth and fifth day after exposure, after the sixth day, reproduction in all crosses was lower than that of control (unexposed couples) (Figure 1). Despite of having two morphologically morphs of fighter and non-fighter males in this species, only one fighter male was observed in the experiment and we did not consider the effect of fighter male treatment on female fecundity.

3.3 Transgenerational effects of imidacloprid on the fecundity of R. robini

Despite the observation that sublethal exposure to different concentrations of imidacloprid led to reproductive stimulatory responses in *R. robini* adult females (F_0), no biological differences were observed in the measured biological characteristics of the offspring (F_1), including egg hatching percentage, embryonic period, and sex ratio. However, increasing the concentration of imidacloprid, increased the duration from egg hatch to adult emergence (Table 2).

3.4 Effects of exposure to imidacloprid on the enzyme activities

Changes in the activity of the detoxifying enzymes in *R. robini* were examined immediately after the 48-h exposure to sublethal concentrations of imidacloprid (at the end of the exposure period) and 48 h after the 48-h exposure. Immediately after exposure period, in comparison with control, the activity of P450s was increased significantly in mites treated with 140 and 280 mg a.i./L imidacloprid, while 48 h after the treatment period, the induction of P450s was observed in mites treated with 280 mg a.i./L imidacloprid. Induction of CCEs was observed immediately after treatment only at a concentration of 70 mg a.i./L and no significant change in the activity of CCEs was observed 48 h after the treatment period. The activity of GSTs immediately after the exposure period was significantely lower at 140, 280 mg a.i./L imidacloprid, while 48 h after treatment, significant induction of GSTs activity was observed at 280 mg a.i./L imidacloprid. The activity of CAT, immediately and 48 hours after treatment, was found significantly higher in the mites treated with different concentrations of imidacloprid compared to the control (Table 3).

3.5 Change in diazinon toxicity after pretreatment with a hormetic dose of imidacloprid

Pretreatment of mites with 140 mg a.i./L dose of imidacloprid as a sublethal hormetic dose, reduced the toxicity of diazinon in the pre-treated mites and doubled the LC_{50} of diazinon (Table 4).

4. Discussion

Neonicotinoids are used against a wide range of pests of horticultural, agricultural, and ornamental crops (Jeschke et al., 2011). These compounds can be metabolized to secondary compounds in the plant or pest, some of which still have insecticidal properties, while others might interfere more subtle in the biological processes of insects (Nauen et al., 1999). Various laboratory studies have highlighted the occurrence of stimulant effects (especially on reproductive performance) in pest arthropods when exposed to low doses of sublethal concentrations of neonicotinoids. In addition, greenhouse and field studies have pointed to the possible positive effects of neonicotinoids on some of the pest population dynamics. High population growth and reproductive stimulation have been recorded in several pest species exposed to sublethal doses of imidacloprid such as aphids (Cutler et al., 2009; Qu et al., 2015). Also, increased fertility has been recorded in *Drosophila suzukii* Matsumura adults sublethally exposed to thiamethoxam (Krüger et al., 2021). In the Asian citrus psyllid, *Diaphorina citri* Kuwayama, exposure to a sublethal concentration of imidacloprid was found to increase the reproductive function and induced stimulatory (hormetic) effects (Chen et al., 2020). On the other hand, a significant decrease in demographic parameters was observed in response to sublethal doses of imidacloprid in *Brevicoryne brassicae* Linnaeus (Lashkari et al., 2007).

In mites, a reduced fecundity has been revealed previously in *Tetranychus urticae* Koch exposed to the neonicotinoids i.e. imidacloprid, thiacloprid, acetamiprid, and thiamethoxam (Ako et al., 2004). However, other studies report a 10-26% increase in the oviposition of *T. urticae* after drench or foliar applications of imidacloprid (James and Price, 2002). Also, a significant increase in the *T. urticae* mite population was observed in the field after foliar application of imidacloprid against aphids (Beers and Himmel, 2002).

Here, we demonstrated that sublethal exposure to three neonicotinoids, i.e. imidacloprid, thiamethoxam, and dinotefuran, affected the fecundity (eggs laid) in *R. robini* females, and the increase in the number of eggs laid by imidacloprid treated females was higher than that of two other neonicotinoids, 24 h after the treatment period. In our study, only dinotefuran treatment did not result in a significant effect on fecundity. This might be associated

with the lower nerve-excitatory activity of dinotefuran compared to imidacloprid. The insecticidal activity of dinotefuran and its derivatives is more closely related to nerve-blocking activity, while nerve-excitatory activity is a characteristic observed in other neonicotinoids (Simon-Delso et al., 2015).

Sublethal exposure to insecticides can lead to reproductive success in male insects (Haddi et al., 2016; Wang et al., 2010) but most often quantification of the stimulatory effects focus on female reproductive performance (Cutler et al., 2009; James and Price, 2002; Santos et al., 2016; Szczepaniec and Raupp, 2013). In this study, it was found that in four different crosses between males and females treated with a hormetic concentration of imidacloprid (70 mg a.i./L), the highest fecundity (eggs laid) in *R. robini* females resulted from the mating where only the female was treated. Our result on the decreased fecundity of untreated females when coupled with imidacloprid-treated males was somehow contradictory. For instance, males of *Euschistus heros* (Fabricius) (Hemiptera: Pentatomidae) exposed to sublethal concentrations of imidacloprid induced higher fecundity when mated to untreated females (Haddi et al., 2016). In the brown planthopper, triazophos treated females when coupled to treated males showed higher fecundity than that of coupled with untreated males, but the percent increase was affected by the temperature (Yu et al., 2012). These reports on enhanced fecundity have been linked to higher walking activity and mating to a greater extent in insecticide-treated males. Future experiments are needed to clarify the potential alterations in mating the behavior of *R. robini* that may result from imidacloprid exposure.

Stimulating effects by sublethal doses of insecticides may affect the biology of the next generation or be passed on through generations. For example, treatment of *Podisus maculiventris* Say with low doses of imidacloprid enhanced fertility, fecundity, and survival at different stages of life and over two generations (Rix and Cutler, 2020). Transgenerational negative impacts have also been demonstrated in the biological performance of *Coccinella septempunctata* Linnaeus exposed to thiamethoxam (Jiang et al., 2019). Stimulatory effects on the pre-adult stage, longevity, and fertility were observed in the progeny generation (F_1) of *A. gossypii* when parental aphids (F_0) were exposed to low doses of thiamethoxam (Ullah et al., 2020). In this study, no significant difference

was observed in the percentage of egg hatching, embryonic period, and sex ratio of the offspring in the female mites treated with imidacloprid. However, the duration from egg hatch to adult emergence was increased with increasing doses of imidacloprid and it was significant at twice the field dose. Therefore, a concentration that stimulates processes in the parent generation may be inhibitory in the next generations. For example, it has been observed that concentrations of insecticides that initially stimulate the reproduction or longevity of aphids have led to reduced reproduction and longevity in subsequent generations (Ayyanath et al., 2013; Ayyanath et al., 2015). Treatment of the mirid bug, *Apolygus lucorum* Meyer-Dür with a sublethal dose of dinotefuran was reported to increase the oviposition period and male adult longevity and to reduce nymphal survival rate in the F_0 generation. It however decreased the duration of egg and preadult stages, and the total preoviposition period of the F_1 generation (Lu et al., 2020).

Sublethal concentrations of pesticides, in addition to their effects on the reproductive behavior and developmental characteristics of the exposed arthropod, may influence the activity of several enzymes through metabolic complex processes (Zhou et al., 2019). Insects and mites transport or metabolize toxic compounds such as insecticides with the help of protective enzymes (Bolter and Chefurka, 1990; Huang et al., 2019). Our results demonstrated that imidacloprid treatment increased the activity of detoxifying enzymes in females of *R. robini*. The highest activity of CCEs and GSTs was observed in the mites treated with 70 mg a.i./L and for P450s with 140 mg a.i./L imidacloprid just following the exposure period. However, negative effects were observed in the study of biological parameters of the offspring of the first generation of treated females. Considering the relationship between the activity of detoxifying enzymes and transgenerational effects, it seems that the negative effects observed in the biological parameters of the F_1 generation are possibly the results of the overexpression of P450 genes and activation of the detoxification pathway (Sanada-Morimura et al., 2019; Wen et al., 2009). Because enzymatic metabolic detoxification requires energy expenditure, it can have negative effects on the life table traits. In *E. heros*, for example, continuous exposure to imidacloprid increased the activity of P450s and reduced nymph survival and adult lifespan (Castellanos et al., 2019). Increased levels of catalase activity revealed

that the mite is trying to minimize the oxidative stress caused by the insecticide and maintain a state of equilibrium. Many studies have shown increased activity of detoxifying and protective enzymes after exposure to neonicotinoid insecticides in other arthropod species (Liu et al., 2014; Ma et al., 2009; Sun et al., 2015; Zhang et al., 2020). This increased enzyme activity may be associated with the induction of reproductive stimulatory effects at the hormetic doses. Exposure to imidacloprid of *Frankliniella occidentalis* Pergande and *F. intense* Trybom has resulted in faster growth and a significant increase in the activity of detoxification enzymes (CCE, P450s, and acetylcholinesterase) and antioxidant enzymes (catalase and peroxidase) in both thrips species (Zhang et al., 2020). These findings, together with conclusions from our study, indicate that the increased activity of these enzymes along with the stimulant effects of reproduction after treatment can contribute to outbreaks of this pest.

The effects of hormesis on the response to insecticide stress may not only cause serious problems for pest management by increased pest outbreaks (Cohen, 2006) but may also enhance rapid adaptation to other stressful environments (Hoffmann and Hercus, 2000). Increased P450s activity can have exacerbating or suppressive effects on the toxicity of organophosphate insecticides (Anderson and Zhu, 2004). In this study, diazinon toxicity was seriously affected (two-fold difference in LC_{50}) by pretreatment with imidacloprid at a hormetic dose of 140 mg a.i./L. At this dose, the activity of P450s also increased significantly. Although more evidence is needed to determine the association between increased P450s activity and decreased susceptibility to diazinon, these results could emphasize the importance of hormetic and sublethal effects of neonicotinoids in the development of potential resistance (Guedes et al., 2010; Guedes and Cutler, 2014; Sial et al., 2018; Ullah et al., 2020).

5. Conclusions

In summary, this study demonstrates that exposure to neonicotinoid insecticides, especially imidacloprid, can induce reproductive stimulatory effects in *R. robini.* This might potentially impact the control of this mite by increasing the detoxification enzyme activities which in turn results in increased tolerance to other pesticides.

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Tables & Figures

Table 1. Mean (± SE) fecundity (cumulative number of eggs per female during 24- 96 h after treatment period) of *R. robini* after treatment with neonicotinoid insecticides.

Insecticide	concentrations	time after treatment (h)			
	mg a.i./L	24	48	72	96
Imidacloprid	0	10.12 ± 0.59 b*	15.09 ± 0.84 a	18.57 ± 1.09 a	21.42 ± 1.26 a
	35 (FD/4)	11.61 ± 0.89 ab	16.45 ± 1.26 a	20.03 ± 1.56 a	22.51 ± 1.71 a
	70 (FD/2)	13.55 ± 0.76 a	18.03 ± 1.36 a	21.75 ± 1.55 a	25.03 ± 1.87 a
	140 (FD)	13.96 ± 1.13 a	16.72 ±1.68 a	20.31 ± 1.96 a	22.15 ± 1.06 a
	280 (2FD)	11.85 ± 0.86 ab	16.60 ± 1.28 a	20.73 ± 1.70 a	24.6 ± 1.96 a
Thiamethoxam	0	9.74 ± 0.56 b	15.5 ± 0.89 ab	20.31 ± 1.32 ab	23.06 ± 1.43 ab
	25 (FD/4)	12.31 ± 0.85 a	17.83 ± 1.21 a	21.86 ± 1.56 a	23.7 ± 1.64 a
	50 (FD/2)	12.93 ± 0.93 a	17 ± 1.33 ab	20.22 ± 1.68 ab	21.87 ± 1.87 ab
	100 (FD)	10.96 ± 1.15 ab	14.56 ± 1.69 ab	17.46 ± 2.11 ab	18.71 ± 2.20 ab
	200 (2FD)	9.43 ± 0.91 b	13.96 ± 1.31 b	16.4 ± 1.50 b	17.96 ± 1.76 b
Dinotefuran	0	7.28 ± 0.39 a	12.03 ± 0.60 ab	15.46 ± 0.80 a	17 ± 0.94 a
	37.5 (FD/4)	7.28 ± 0.47 a	11.68 ± 0.65 ab	14.43 ± 0.83 ab	15.59 ± 0.88 ab
	75 (FD/2)	7.23 ± 0.40 a	10.68 ± 0.68 b	12.62 ± 0.85 b	13.62 ± 0.94 b
	150 (FD)	8.64 ± 0.66 a	12.90 ± 0.83 a	14.96 ± 0.90 ab	15.37 ± 1.04 ab
	300 (2FD)	7.90 ± 0.57 a	11.53 ± 0.68 ab	13.93 ± 0.86 ab	14.84 ± 0.93 ab

* Means within a column followed by the same letter are not significantly different at $P < 0.05$ with LSD test.

Table 2. Mean ± SE of egg hatch rate, duration of the embryonic period, duration from egg hatch to adult emergence, and sex ratio of *R.robini* treated with imidacloprid in F_1.

doses	Hatch rate of eggs (%)	Embryonic period (days)	Development time (days)	Sex ratio (%females)
0	97.38 ± 1.31a*	4.67 ± 0.10a	10.95 ± 0.35 bc	60.83 ± 9.38 a
35	94.42 ± 1.59a	4.61 ± 0.09a	10.41 ± 0.43 c	68.05 ± 11.72 a
70	97.59 ± 0.44a	4.53 ± 0.08a	11.45 ± 0.40 abc	41 ± 6.98 a
140	96.57 ± 1.50a	4.51 ± 0.05a	12.43 ± 0.55 ab	58.33 ± 12.5 a
280	96.55 ± 0.25a	4.67 ± 0.10a	12.63 ± 0.70 a	48.21 ± 1.54 a
F values	0.92	0.55	3.2	0.7
P >F	0.4763	0.703	0.0157	0.605

* Means within a column followed by the same letter are not significantly different at P < 0. 05 with LSD test.

Table 3. Detoxification and protective enzyme activities in *R.robini* treated with different concentrations of imidacloprid.

Time after exposure period (h)	Doses	Enzyme activity			
		P450 (Unit/mg protein)	CCE (nmol/min/mg protein)	GST (nmol/min/mg protein)	CAT (μmol/min/mg protein)
0 h	control	12.25±0.43 b*	2168.62±74.11 b	134.36±13.15 a	16.78±1.42 b
	35	11.52±0.31 b	2269.35±76.29 ab	122.35±2.55 a	24.47±2.43 a
	70	12.21±0.80 b	2534.54±103.90 a	141.14±1.69 a	23.81±1.04 a
	140	17.80±1.75 a	2074.81±63.64 b	87.61±0.82 b	19.85±0.70 ab
	280	18.45±1.88 a	2061.61±143.99 b	93.67±0.86 b	21.88±0.32 a
48 h	control	24.41±1.75 b	2911.57±89.43 a	52.82±6.29 b	18.95±0.28 c
	35	24.26±1.51 b	2850.14±144.05 a	50.08±0.47 b	33.72±1.16 a
	70	18.52±0.17 c	2705.10±142.63 a	58.66±4.09 ab	23.68±0.58 b
	140	21.73±0.99 bc	2467.74±123.78 a	58.71±2.12 ab	20.96±0.80 bc
	280	41.48±0.43 a	2872.74±149.07 a	75.50±8.73 a	23.70±0.85 b

* Means within a column followed by the same letter are not significantly different at $P < 0.05$ with LSD test.

Table 4. Log-dose probit-mortality data of diazinon in *R. robini* female mites pre-treated with distilled water or imidacloprid (140 mg a.i. L^{-1})

Pre-treatment	LC_{50} (mg a.i. L^{-1})	Confidence interval (95%)	$\chi 2$ (df)	Slope ± SE
Distilled water	0.316	0.237-0.374	45.92(14)	0.49±4.42
Imidacloprid	0.633	0.528-0.707	25.13(14)	1.34±7.88

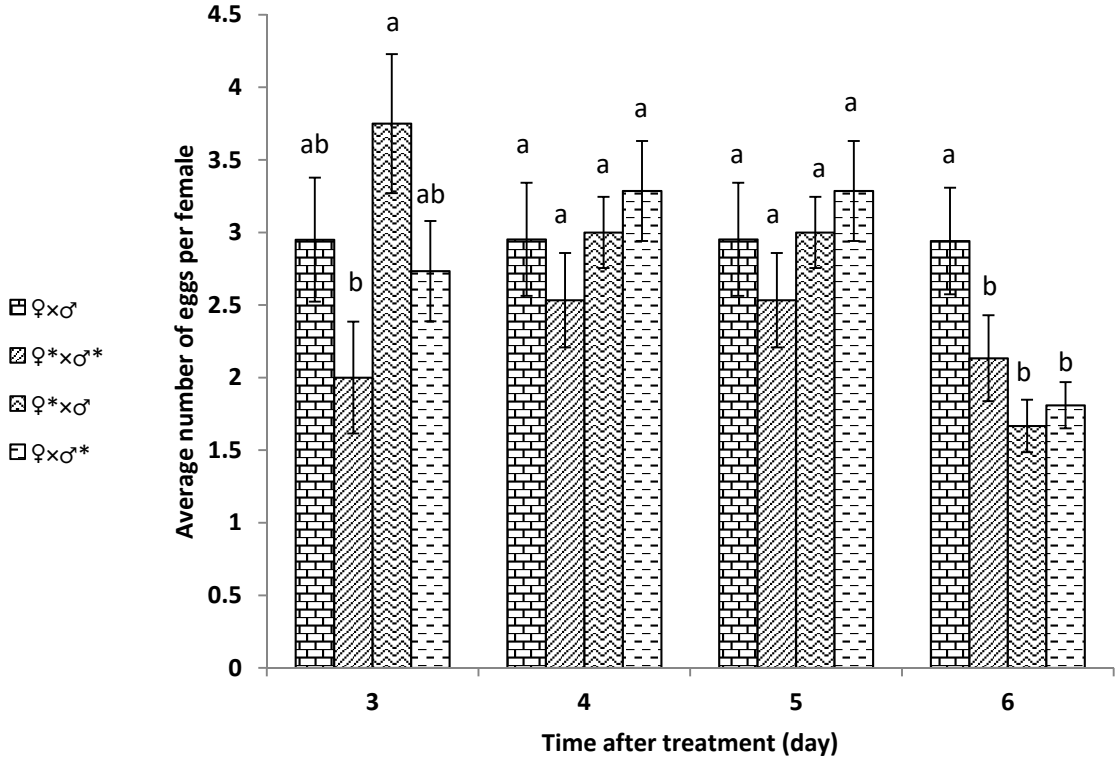

Fig 1. Effect of exposure of different *R. robini* sexes to imidacloprid on the fecundity of females. The asterisk symbol indicates the sex being treated. Bars with different letters indicate that means differ significantly at $P < 0.05$ with LSD test.