Pesticide Biochemistry and Physiology 135 (2017) 59–63

Contents lists available at ScienceDirect

Pesticide Biochemistry and Physiology

journal homepage: www.elsevier.com/locate/pest

Resistance of green lacewing, *Chrysoperla carnea* Stephens to nitenpyram: Cross-resistance patterns, mechanism, stability, and realized heritability

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ARTICLE INFO

Article history:
Received 21 January 2016
Received in revised form 12 April 2016
Accepted 18 June 2016
Available online 20 June 2016

Keywords:
Indigenous predator
Neonicotinoids
Resistance evolution
Cross-resistance
Biochemical mechanism

ABSTRACT

The green lacewing, *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) is a major generalist predator employed in integrated pest management (IPM) plans for pest control on many crops. Nitenpyram, a neonicotinoid insecticide has widely been used against the sucking pests of cotton in Pakistan. Therefore, a field green lacewing strain was exposed to nitenpyram for five generations to investigate resistance evolution, cross-resistance pattern, stability, realized heritability, and mechanisms of resistance. Before starting the selection with nitenpyram, a field collected strain showed 22.08-, 23.09-, 484.69- and 602.90-fold resistance to nitenpyram, buprofezin, spinosad and acetamiprid, respectively compared with the Susceptible strain. After continuous selection for five generations (G1–G5) with nitenpyram in the laboratory, the Field strain (Niten-SEL) developed a resistance ratio of 423.95 at G6. The Niten-SEL strain at G6 showed no cross-resistance to buprofezin and acetamiprid and negative cross-resistance to spinosad compared with the Field strain (G1). For resistance stability, the Niten-SEL strain was left unexposed to any insecticide for four generations (G6–G9) and bioassay results at G10 showed that resistance to nitenpyram, buprofezin and spinosad was stable, while resistance to acetamiprid was unstable. The realized heritability values were 0.97, 0.16, 0.03, and −0.16 to nitenpyram, buprofezin, acetamiprid and spinosad, respectively, after five generations of selection. Moreover, the enzyme inhibitors (PBO or DEF) significantly decreased the nitenpyram resistance in the resistant strain, suggesting that resistance was due to microsomal oxidases and esterases. These results are very helpful for integration of green lacewings in IPM programs.

1. Introduction

Generalist predators have played a key role in controlling various pests on crops [1,2]. The green lacewing, *Chrysoperla carnea* Stephens is one of the general predators used in different cropping systems [3,4]. Green lacewings play a vital role in pest control due to their capability of controlling a wide range of insect pests, high searching capacity, and high tolerance to ecological factors compared to that of other predators [5–7]. This predator is the most commonly used and widely spread in whole Holarctic. The green lacewing is an important predator in those cropping systems where insecticide applications are used for pest management as a main tool, such as in Pakistan [6–8]. It is an effective predator used for the control of mites, whiteflies, thrips, mealy bugs, aphids, and caterpillars [7,9,10].

Cotton, *Gossypium hirsutum* L. is a cash crop which plays a key role in the agricultural financial systems of Pakistan. Cotton is basically grown for fiber, but cotton seeds are a major source of oil for humans and seed cake for domestic livestock. It provides employment (ginning, textile and edible oil business) to millions of people in cotton based business and on farm [11]. In Pakistan, cotton production is reduced compared with numerous developed countries due to attack by various sucking and chewing pests, such as whitefly (*Bemisia tabaci* Genn.), aphids (*Aphis gossipii* Glov.), thrips (*Thrips tabaci*), jassid (*Amrasca bigutella* Ishida.), mealybug (*Phenacoccus solenopsis* Tinsley), pink bollworm (*Pectinophora gossypiella* Saund.), armyworm (*Spodoptera litura* F.), spotted bollworm (*Earias insulana* Boisd.) and American bollworm (*Helicoverpa armigera* Hüb.) [12–16]. These pests cause 20–40% losses to cotton yield in Pakistan [17].

Growers mostly apply chemicals with inappropriate doses and spray methods without asking entomologists to manage various agricultural pests [12]. In the early 1980s, insecticides seemed very successful at controlling sucking and chewing insect pests of cotton and other crops

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http://dx.doi.org/10.1016/j.pestbp.2016.06.004
0048-3575/

[18]. However, due to excessive spray of insecticides, the evolution of resistance by insect pests is a major threat to crop safety and disease transmission [14,19]. Resistance to insecticides has been reported in many agriculturally and medically important insect pests from Pakistan [12–14,20–24], challenging the chemical control for the management of these pests. Moreover, misuse of pesticides has produced pesticide residues in food chain, disappearance of wildlife and resurgence of several secondary pests [25]. Green lacewing is a common predator than other predators that has developed resistance to pyrethroid, organophosphate and biorational insecticides [3,6,26,27], which make them compatible with the different IPM systems. The predators can develop resistance either by consuming other insects that have been exposed with insecticides or by direct contact with insecticides [28].

The major mechanism involved in neonicotinoid resistance is metabolic detoxification with increase in cytochrome P450 and esterases in many insect pests [29–31]. However, the mechanism of nitenpyram resistance in green lacewing is not reported worldwide. Green lacewings are cited as the first line of protection in IPM systems [26]. Neonicotinoids (e.g. nitenpyram) are being extensively used in Pakistan for the control of various sucking insect pests of cotton, ornamentals and vegetables [32]. Therefore, the assessment of resistance evolution, cross-resistance, mechanism, stability and realized heritability of resistance to nitenpyram is necessary in green lacewing, to make it compatible in diverse IPM systems [26]. For this purpose, a population of green lacewings from Muzaffargarh, Pakistan was collected and selected with a commonly used neonicotinoid insecticide, nitenpyram, in the laboratory to study the aforementioned parameters.

2. Materials and methods

2.1. Insect collection and rearing

Green lacewing adults (about 300) were collected from cotton fields of Muzaffargarh (location: 30.0703° N, 71.1933° E) in 2014, by using plastic vials and designated as Field strain. These adults were kept in a rearing cage (23 × 38 × 38) provisioned with artificial diet containing distilled water, honey, and yeast with a ratio of 4:2:1, respectively, in the laboratory [3]. Black glossy papers were suspended for egg laying in rearing cages and were removed on an alternate day to collect the eggs. These eggs were kept in locally made plastic capsules. After hatching (2–3 days), each single larva was transferred to Petri dishes (5 cm in diameter) and fed on frozen Angoumois grain moth, *Sitotroga cerealella* Olivier eggs [3,6]. The eggs of Angoumois grain moth were provided after 48 h and were provided till pupation. The culture was maintained in the laboratory at 26 ± 2 °C temperature, 65 ± 5% RH and 14:10 h (L:D) photoperiod.

The population of green lacewings collected from Multan in 1999, and reared for 15 years without insecticide exposure, was previously designated as Susceptible strain [3,6,26].

2.2. Insecticide formulations and enzyme inhibitors

Commercial insecticides were used for bioassays. Insecticides included; nitenpyram (Paranol 10EC, Agrochemicals), buprofezin (Fuzin 25WP, Four Brothers), and acetamiprid (Mospilan 20WP, Dow AgroSciences); biological insecticides included spinosad (Tracer 240SC, Arysta Life Sciences); and enzyme inhibitors included PBO (Piperonyl butoxide; Sigma Ltd., UK), an inhibitor of cytochrome P450 monooxygenases and esterases, and DEF (*S*,*S*,*S*-tributyl phosphorotrithioate, Sigma Ltd., UK), an esterase specific inhibitor.

2.3. Selection experiment

The Field strain was separated into two further sub-strains in the first generation (G1); one was exposed to nitenpyram for five generations (G1–G5), designated as Niten-SEL and second one was left unexposed for five generations, designated as UNSEL. The field-collected population (G1) was bioassayed to know the lethal concentrations (LCs) for desired selection process (for example, LC_{50}, LC_{70}, LC_{85}, LC_{90}, and LC_{95}) before starting experiment. First instar larvae were topically exposed to different concentrations of nitenpyram (Table 1) for five generations by using a handheld micro applicator for selection experiment. The solution of nitenpyram (0.5 μl) was applied on the thorax of each larva as described by Abbas et al. [6]. About 300 larvae per generation were exposed for each selection. The exposed larvae were placed into Petri dishes (5 cm) and fed Angoumois grain moth eggs until pupation. The emerging adults were the parents of the next generation.

2.4. Concentration response bioassay

Concentration response bioassays of insecticides were conducted on two to three day old green lacewing larvae, as described previously [3,6]. Four insecticide concentrations were made as serial dilutions. Each concentration was replicated four times for each bioassay. The serial concentrations of the insecticides were applied on the thorax of each individual larva as described by Abbas et al. [6]. In each replication, twenty larvae were exposed, for a total of eighty larvae for each concentration of insecticide. For control, thirty larvae were exposed to water only. Treated larvae were supplied with eggs of Angoumois grain moths. All bioassays were kept at laboratory conditions as mentioned above. Mortality was assessed 72 h after exposure of insecticides.

2.5. Synergism analyses

The enzyme inhibitors, PBO or DEF, were diluted first in acetone (Analytical reagent, Fisher Scientific, Lough borough, UK), then mixed with insecticide concentrations. PBO or DEF at 5 mg/ml were used for the Niten-SEL and Susceptible strains. For the control, acetone alone was used. Mortality was assessed 72 h after exposure to insecticide. Synergism ratio was calculated as LC_{50} of nitenpyram alone/LC_{50} of nitenpyram + PBO or DEF.

2.6. Stability of resistance

For the stability of resistance, the Niten-SEL strain was left unexposed for four generations (G6–G9). The four generations' unexposed strain was bioassayed again with nitenpyram at G10 to determine whether resistance to nitenpyram and other insecticides was stable or not. A reduction in resistance to insecticides was calculated as follows [33].

$$DR = (\text{logfinal } LC_{50} - \text{loginitial } LC_{50})/n$$

In above equation, *n* is the number of generations left unexposed to nitenpyram.

2.7. The realized heritability (h^2)

The realized heritability was estimated according to Tabashnik [34] and Abbas et al. [35] as h^2 = Response to selection (R)/Selection differential (S). Response to selection was calculated as R = [logfinal LC_{50} of Niten − SEL strain − loginitial LC_{50} of Field strain]/n.

Table 1
Selection history of nitenpyram to the Field strain of green lacewings for five generations.

Generation	Concentration	Number of larvae exposed	Number of larvae killed	Survival (%)
G1	36.23	320	206	35.62
G2	50.00	300	162	46.00
G3	77.88	300	133	55.67
G4	93.34	300	95	68.33
G5	122.07	300	78	74.00