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Risk assessment, cross-resistance potential, and biochemical mechanism of resistance to emamectin benzoate in a field strain of house fly (*Musca domestica* Linnaeus)

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HIGHLIGHTS

- Rapid development of resistance to emamectin was observed as a result of selection experiments.
- The EB-SEL strain showed reduction in resistance to abamectin, indoxacarb and thiamethoxam.
- Metabolic resistance mechanism was not responsible in developing emamectin resistance in the EB-SEL strain.

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ABSTRACT

Reduced sensitivity to insecticides in insect pests often results in control failures and increases in the dose and frequency of applications, ultimately polluting the environment. Reduced sensitivity to emamectin benzoate, a broad-spectrum agrochemical belonging to the avermectin group of pesticides, was reported in house flies (*Musca domestica* L.) collected from Punjab, Pakistan, in 2013. The aim of the present study was to investigate the risk for resistance development, biochemical mechanism, and cross-resistance potential to other insecticides in an emamectin benzoate selected (EB-SEL) strain of house flies. A field-collected strain showing reduced sensitivity to emamectin was re-selected in the laboratory for five consecutive generations and compared with a laboratory susceptible (Lab-Susceptible) reference strain, using bioassays. The field strain showed rapid development of resistance to emamectin (resistance ratio (RR) increased from 35.15 to 149.26-fold) as a result of selection experiments; however, resistance declined when the selection pressure uplifted. The EB-SEL strain showed reduction in resistance to abamectin, indoxacarb, and thiamethoxam. The results of synergism experiments using piperonyl butoxide (PBO) and *S,S,S*-tributylphosphorotrithioate (DEF) enzyme inhibitors and biochemical analyses revealed that the metabolic resistance mechanism was not responsible in developing emamectin resistance in the EB-SEL strain. In conclusion, the risk for the rapid development of emamectin resistance under continuous selection pressure suggests using a multifaceted integrated pest management approach for house flies. Moreover, the instable nature of emamectin resistance in the EB-SEL strain and lack of cross-resistance to other insecticides provide windows for the rotational use of insecticides with different modes of action. This will ultimately reduce emamectin selection pressure and help improving management programs for house flies without polluting the environment.

1. Introduction

The failure to control insect pests of economic importance in the past few decades has largely been due to over-reliance on insecticides in Pakistan. This has resulted in the development of

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reduced sensitivity and resistance to insecticides from different classes and destruction of non-target organisms and natural enemies (Afzal and Shad, 2015). Over-reliance on insecticides has also resulted in deteriorating public and environmental health, necessitating the development of integrated approaches for several insect pests including the house fly, *Musca domestica* L., (Diptera: Muscidae) (Khan et al., 2013a). The house fly is a major pest of dairies with the potential to transmit a number of medical and veterinary pathogens. Dairies play an important role in the spread of house flies owing to the abundance of animal manure, favourable temperature, and humidity (Khan et al., 2012). For this reason, the use of insecticides, a quick control measure, has been the choice of the majority of dairy producers to control the spread of house flies. However, resistance to several insecticides from different classes has been reported around the globe (Kaufman et al., 2006; Memmi, 2010; Khan et al., 2013a, b). Dairy producers therefore face many difficulties controlling house fly populations. They usually only have the option to increase the dose or frequency of pesticide sprays. In the Punjab province of Pakistan, dairy producers usually control house flies with a variety of insecticides from different classes, such as organophosphate, carbamate, pyrethroid, and new chemicals (Khan et al., 2013c). Resistance to some of the insecticides from these classes was reported in 2013–14 (Khan et al., 2013a, b), likely a product of their excessive use.

Emamectin benzoate is a broad-spectrum pesticide with a novel mode of action. The insecticide is derived from naturally occurring avermectin family of compounds, all of which exhibit toxicity for several pests including arthropods (Lumaret et al., 2012). It's mode of action in insects includes the activation of chloride channels in the nervous system, which prevent muscle contraction, causing the insect to stop feeding causing death (Ishaaya, 2002). Avermectins have been strongly recommended for the management of different dairy pests in Punjab, Pakistan (Muhammad et al., 2008). Recently, notable levels of field-evolved resistance to avermectins, including emamectin benzoate, have been reported in different field strains of house flies (Khan et al., 2013b). However, information is lacking on the rapidity with which emamectin resistance can develop under selection pressure, the mechanism of resistance development, and cross-resistance potential. Therefore, the objectives of the present study were to explore the risk for the development of emamectin resistance through selection experiments and to identify possible biochemical mechanisms and cross-resistance potential.

2. Methodology

The methodology followed in the present study has been adapted from Khan et al. (2015a, b) with some modifications. Briefly, a susceptible reference strain (Lab-susceptible) and a resistant field-collected strain (G0) of house flies were maintained in the laboratory as described previously (Khan et al., 2014). The field strain was collected from a dairy farm near Lahore (31° 32′ 59 N; 74° 20′ 37 E), Punjab, Pakistan, and was re-selected in the laboratory with emamectin benzoate. Four technical-grade insecticides (Chem Service Inc, West Chester PA): abamectin, emamectin benzoate, indoxacarb, and thiamethoxam were used for toxicological evaluations. In addition, two synergists, *S,S,S*-tributylphosphorotrithioate (DEF) and piperonyl butoxide (PBO), were also used in synergism studies. A no choice feeding bioassay method (IRAC method # 026) was used in the selection experiments and insecticidal bioassays. The selection experiments were performed by exposing newly emerged (<one-day-old) unmated house flies to emamectin benzoate for five consecutive generations (G1-G5) and the strain was named emamectin benzoate selected (EB-SEL) strain. The mortality data were recorded after 48 h of exposure of flies to emamectin benzoate and the survivors were used as the parents of the next generation. After the selection experiments, flies from both of the strains (Lab-susceptible and EB-SEL) were used in synergism experiments and enzyme analyses for the purpose to study the mechanism of resistance to emamectin benzoate. Full details of the methodology regarding toxicological evaluations, selection experiments and data analyses are presented in Khan et al. (2015a), whereas the details of enzyme analyses are presented in Khan et al. (2015b) (the details are also provided as supplementary materials and methods).

3. Results

The results of the bioassays against the Lab-susceptible reference and field strains (G0) revealed varying responses to different insecticides (Table 1). For the Lab-susceptible reference strain, abamectin was the most toxic insecticide, followed by indoxacarb with LC_{50} values of 0.83 and 1.36 ppm, respectively. This difference was statistically significant. Emamectin benzoate and thiamethoxam were least toxic and statistically equivalent. For the newly collected field strain (G0), indoxacarb was the most toxic insecticide followed by thiamethoxam, emamectin, and abamectin. Before starting the selection experiments, the field strain (G0) in comparison to the Lab-susceptible reference strain displayed considerable resistance to abamectin (134.28-fold), emamectin (35.15-fold), thiamethoxam (6.49-fold), and indoxacarb (5.48-fold).

The field strain (G0) showing resistance to emamectin (35.15-fold) before the selection experiment was re-selected with emamectin in the laboratory for five consecutive generations and named emamectin benzoate selected (EB-SEL) strain. The results of the bioassays after the selection experiments revealed a notable development of emamectin resistance (149.26-fold; Table 2). However, emamectin resistance declined from 149.26- to 58.78-fold when the emamectin selection pressure was lifted for an additional five generations.

Reduction in resistance to other insecticides tested has been observed in the EB-SEL strain. Before the selection experiment, the field strain (G0) showed notable resistance to abamectin (134.28-fold), indoxacarb (5.48-fold), and thiamethoxam (6.49-fold). However, after the selection experiment, resistance in the EB-SEL strain declined to all these insecticides i.e., 134.28–47.49- fold for abamectin, 5.48–1.68 fold for indoxacarb and 6.49–3.61-fold for thiamethoxam (Table 3).

Synergistic effects of enzyme inhibitors on the toxicity of emamectin to the Lab-susceptible reference and the EB-SEL strain revealed that the administration of PBO and DEF with emamectin in bioassays did not significantly enhance the toxicity in the studied strains (overlapping 95% CIs) (Table 4). Moreover, the activities of CarE and MFO in both of the studied strains were statistically non-significant ($P > 0.05$; Table 5), suggesting that other mechanisms than metabolic resistance may be responsible for the development of resistance.

4. Discussion

Notable levels of resistance to emamectin, abamectin, indoxacarb, and thiamethoxam were found in the field-collected house fly strain. In previous studies (Khan et al., 2013b, 2015b), we have shown significant levels of resistance to these insecticides in field strains of house flies collected from different areas, and the results of the present experiments have further strengthened the findings of resistance to emamectin, abamectin, indoxacarb, and thiamethoxam. The most probable reason for the occurrence of resistance to the above mentioned insecticides is the fact that the field strain was collected from an open-structured dairy farm with intensive