Pesticide Biochemistry and Physiology 111 (2014) 24–30

Contents lists available at ScienceDirect

Pesticide Biochemistry and Physiology

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

Resistance selection and biochemical mechanism of resistance against cyflumetofen in *Tetranychus cinnabarinus* (Boisduval)

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ARTICLE INFO

Article history:
Received 11 October 2013
Accepted 7 April 2014
Available online 18 April 2014

Keywords:
Tetranychus cinnabarinus
Cyflumetofen
Resistance selection
Glutathione-S-transferases
Carboxylesterase
Mixed function oxidase

ABSTRACT

The carmine spider mite, *Tetranychus cinnabarinus* is an important crop and vegetable plants pest mite. As a novel acaricide, cyflumetofen is effective against *Tetranychus* and *Panonychus* mites, but its risk and biochemical mechanism of resistance in mites is not clear. In this study, the resistance against cyflumetofen was selected and its biochemical mechanisms were studied in *T. cinnabarinus*. After selection the susceptibility and resistance against cyflumetofen in *T. cinnabarinus*, the final resistance ratio reached 21.33 at LC_{50} (CyR_{-43}/CyS). All the collected field populations showed low resistance against cyflumetofen, although it had never been used in China. The activity of detoxifying enzymes CarE, MFO and GSTs were significantly increased in the final selected resistance strain (CyR_{-43}), especially that for GSTs increased more than 7-folds after selection. The resistance against cyflumetofen developed slowly when selected from the susceptible strain in laboratory, but the resistant genes already existed in field populations, and the GSTs was the most important detoxifying enzyme conferring resistance against cyflumetofen in *T. cinnabarinus*. These results would provide the valuable information for designing appropriate strategies for the practical application of cyflumetofen in the field and delaying resistance development.

1. Introduction

The carmine spider mite, *Tetranychus cinnabarinus* (Boisduval) (Acarina: Tetranychidae), is one of the most important pest species responsible for significant yield losses, it enjoys worldwide distribution with more than 100 crops or plants grown in the field or greenhouse, just like beans, aubergines, peppers, tomatoes, cucurbits and many other crops [1–3]. In recent years, the intensive use of insecticides and acaricides has led to resistance in many insects and mite species around the globe, their control becomes exceedingly challenging [4–6]. Due to the mites' short life cycle, a restricted area of activity with a short maturation period, a high reproductive rate and been frequently exposed to various insecticides and acaricides, the *Tetranychus* mites, including *T. cinnabarinus* and its sibling species *Tetranychus urticae* , have a higher level of resistance compared with insects [7–11]. In fact, *T. urticae* has the highest incidence of pesticide resistance among arthropods [12].

Cyflumetofen is a novel acaricide, which was first registered in Japan by 2007 and began to use for mite control in many countries. It is extremely effective against *Tetranychus* and *Panonychus* mites, and active against other phytophagous mites, while quite safe to predatory phytoseiidae mites and other non-target organisms [13–16]. At the same time, no phytotoxicity has been found on varieties of crops [17]. Nowadays, there are already many studies on synthesis of cyflumetofen [16,18–20], however, its risk and mechanism of resistance in mites are unknown. The rational development of resistance management strategies for newly developed insecticides may not be possible without identification of mechanisms conferring resistance to particular insecticides. In many arthropod pests, the development of insecticide resistance is closely linked with increases in the activity of various detoxifying enzymes inside their bodies [21], which mainly include glutathione-S-transferases (GSTs), carboxylesterase (CarE) and mixed function oxidase (MFO). They all play important roles in the resistance formation of many pests and detoxification metabolism of exogenous compounds.

The present study reports the results on resistance selection and biochemical mechanism of resistance against cyflumetofen in *T. cinnabarinus*, which would benefit the management of the cyflumetofen resistance in mites.

2. Materials and methods

2.1. Strains

2.1.1. Laboratory strain

T. cinnabarinus collected in the field of Beibei District, Chongqing, China, were transferred to fresh potted young cowpea plants.

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http://dx.doi.org/10.1016/j.pestbp.2014.04.004
0048-3575/

The 200th generation after about 13 years' breeding indoor under artificial climate and pesticide free was considered relatively susceptible strain (SS).

2.1.2. Field strains

There were 6 field strains, which were collected from Tongnan county (TN), Bishan county (BS), Wulong county (WL) (The above three counties belong to Chongqing municipality directly under the Central Government, China), Gejiu city, Yunnan province (GJ), Jinzhou city, Hubei province (JZ) and Hangzhou city, Zhejiang province (HZ), respectively. All the field strains were maintained in laboratory culture for two generations without further selection by acaricides.

All the mites were cultivated at 26 ± 1 °C and 55–70% humidity with 14 h light exposure (8 × 40 W solar lamps) and 10 h darkness.

2.2. Reagents

The 98.8% cyflumetofen and 20% cyflumetofen SC were from Hangzhou Fumeite Plant Protective Ltd. (Hangzhou, China); 97% piperonyl butoxide (PBO) was from Sigma–Aldrich Co. (Saint Louis, USA); the 97% diethyl maleate (DEM) was from Sichuan Xiya Chemical Company Inc. (Chengdu, China); the 97% S,S,S-Tributyl phosphorotrithioate (DEF) was from ChemService Company Inc. (Chester, USA); α-naphthol was from Shanghai Chemical Reagent Company of Chinese Medical Group (Shanghai, China); 1-chloro-2,4-dini-trobenzene (CDNB) was from the Shanghai No.1 Reagent Factory (Shanghai, China); α-naphthyl acetate (α-NA) and β-naphthyl acetate (β-NA) were from Shanghai Qingpu Synthetic Reagent Factory (Shanghai, China); Fast blue B salt, Fast blue RR salt and Bromophenol blue were from the Shanghai Equilibrated Reagent Factory (Shanghai, China); physostigmine was from Fluka (Buchs, Switzerland); Coomassie blue G-250 was from Amresco Co.(Solon, USA); bovine serum albumin (BSA) was from Shanghai BioLife Science & Technology Co. (Shanghai, China); sodium dodecyl sulfate (SDS) was from Sigma (Saint Louis, Missouri, USA); Reduced gluta-thione was from Shanghai Yeast Factory (Shanghai, China); p-nitrophenol and p-nitroanisole were from Shanghai SSS Reagent Co.(Shanghai, China); coenzyme NADPH and hydroxymethyl aminomethane (Tris) were from Shanghai Dingguo Biotech Development Co. (Shanghai, China); sucrose and Triton X-100 were from Amresco Co. (Solon, USA); ammonium persulphate (APS) was from Xian Chemical Company Inc. (Xian, China); N,N′-Methylene diacrylamide (Bis) was from Beijing Chemical Company Inc. (Beijing, China); acrylamide (Acr) and disodium (EDTA-Na_2) were form Beijing chemical reagent Co. (Beijing, China); and N,N,N′,N′-tetramethyl ethylenediamine (TEMED) was from Merck (Darmstadt, Germany).

2.3. Bioassay

Median lethal concentration (LC_{50}) values were measured using the modified residual coated vial (RCV) method recommended by Van Leeuwen. [22–24], detailed information on the bioassay procedure was given by Feng. [25], in which cyflumetofen was dissolved in acetone to at least 7 concentrations (0.5–30 mg L^{-1}) to keep mortality at 20–80%. Thirty 3–5 days old healthy adult females were transferred into the cyflumetofen-coated centrifuge tube, each dose was performed in three replicates. The mites were checked under anatomical microscope after 24 h treatment. Mites showing immobility or with legs irregularly trembling were considered dead. In order to investigate the roles of detoxifying enzymes (GSTs, CarEs and MFOs) in conferring resistance against cyflumetofen, three synergists (PBO, an inhibitor of P450s, DEF, an inhibitor of esterase, and DEM, an inhibitor of GSTs) and their mixtures were adopted in synergism bioassay, which were respectively carried out in 28 and 45 generations (CyR_{-28} and $Cyr_{-45}(CyR_{-43})$). $Cyr_{-45}(CyR_{-43})$ meant that the resistant level in generation 45 was equal to that in generation 43, because the last resistance selection was carried out in generation 43 (CyR_{-43}) and the generation 45 (Cyr_{-45}) was the descendent from CyR_{-43} without further selection and their resistant levels were supposed to be about the same. When conducting a synergism bioassay, the three synergists and cyflumetofen were first mixed in the ratio suggested by He [26], i.e. synergists: cyflumetofen = 3:1 (m/m) (PBO 0.031 g:cyflumetofen 0.01 g, DEF 0.031 g:cyflumetofen 0.01 g, DEM 0.031 g:cyflumetofen 0.01 g and PBO 0.01 g + DEF 0.01 g + DEM 0.01 g:cyflumetofen 0.01 g). The mixtures were subsequently dissolved with 100 mL acetone, respectively. Before use, the mixtures were diluted to various concentrations. Then, its LC_{50}s against *T. cinnabarinus* were measured according to the RCV method described above. Mites treated with synergists only served as the control.

Standard probability value analysis was carried out for the data obtained by the toxicity test according to Finney's method [27].

2.4. Resistance selection

For selecting the resistance strain against cyflumetofen (CyR_{-x} strain, x represented the number of selected-generation) under laboratory conditions, at least 3000 adults female mites were isolated from relatively susceptible strain (SS) and reared on fresh potted young cowpea plants, which were named as CyR_{-0} strain. Cyflumetofen (20% SC), which was diluted 20000-times with water (v/v), was sprayed on the cowpea leaves to exert a selection stress that killed about 70% of the CyR_{-0} and the survivals were transferred to another fresh young cowpea leaves (24 h after spraying), then moved away after 1–2 days' oviposition. The next selecting cycle was conducted after the strain increased. In order to maintain the selection pressure, the concentration of cyflumetofen should be increased after several generations of treatment. Every four or five generations later, toxicity test was carried out to calculate the LC_{50} with RCV method, so that we could keep our eyes on the development trend of cyflumetofen resistance. Detailed information on the selection procedure and rearing conditions was given by He [26].

2.5. Susceptibility selection

The purpose of selection for susceptibility was to remove the gene responsible for cyflumetofen resistance, and then to produce a strain more susceptible to the acaricide (CyS). The method for susceptibility selection was refered to Sato et al. [28] and modified. Briefly, cowpea leaf discs (4.0 cm diameter) were placed upside down on wet cotton pads (8.0 cm diameter, 2 cm height) in petri dishes (10.0 cm diameter). Healthy adult female mites (3–5 days old) from SS strain were transferred singly to each disc, after the adults laid eggs for 2 days, the mites were transferred into the cyflumetofen-coated centrifuge tubes with the concentration of LC_{10} of SS strain (0.59 mg L^{-1}) individually, 24 h later, check the mortality and only progeny of dead females were transferred to fresh young cowpea leaf together and used to produce the next generation, i.e. the CyS strain.

2.6. Determination of protein concentration of enzyme source

Total protein content of the enzyme solution was determined by the Bradford method using bovine serum albumin as the standard [29]. The standard curve was firstly set using bovine serum albumin (BSA) as abscissa, and OD value as ordinate. The 50 μL working enzyme solution was mixed with Coomassie blue, the control was replaced with 0.1 mol L^{-1} pH 7.0 Phosphatic buffer solution (PBS), followed by incubation at 37 °C for 10 min and measure OD value