Crop Protection 94 (2017) 173–177

Contents lists available at ScienceDirect

Crop Protection

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

Mechanism of resistance to pirimicarb in the cowpea aphid *Aphis craccivora*

Mohammed A. Kandil [a], Ibrahim S. Abdallah [a, *], Hala M. Abou-Yousef [b], Naglaa A. Abdallah [c], Eman A. Fouad [b]

[a] Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University, Egypt
[b] Department of Bioassay, Central Pesticides Laboratory, Agriculture Research Center, Giza, Egypt
[c] Department of Genetics, Faculty of Agriculture, Cairo University, Egypt

ARTICLE INFO

Article history:
Received 13 May 2016
Received in revised form
17 December 2016
Accepted 19 December 2016

Keywords:
Detoxifying enzymes
Synergists
Real time PCR

ABSTRACT

A resistant strain of *Aphis craccivora* Koch was selected with pirimicarb for 12 generations. The selected strain exhibited 47-fold resistance compared to the susceptible laboratory strain. The resistant strain also showed cross-resistance to carbosulfan, malathion, chlorpyrifos methyl and thiamethoxam. Low resistance to fenitrothion and acetamiprid was also observed. Bioassays in combination with biochemical synergist studies revealed that the higher inhibition of carboxylesterase using the synergist tributyl phosphorotrithioate (DEF) was associated with increased toxicity of pirimicarb in the resistant strain. On the other hand, piperonyl butoxide (PBO) and diethyl maleate (DEM) had less inhibitory effect on mixed function oxidases (MFO) and glutathione-S-transferase (GST) in the resistant strain. The activity of carboxylesterase was 29-fold greater in the resistant strain, whereas the activity of MFO and GST in the resistant strain was only 5.5 and 1.7- fold greater, respectively. The activity of acetylcholine esterase (AChE) in the resistant strain was 2 fold higher than in the susceptible strain. The I_{50} (the concentration of pirimicarb that inhibit 50% of the enzyme activity) ratio of R-strain to S-strain was 12.6. Molecular studies using real-time quantitative PCR showed that the transcription level of *Ace2 gene* in the resistant strain was 3.4-fold higher than that in the susceptible strain. In conclusion, mechanism of resistance in the pirimicarb resistant strain of *A. craccivora* may include overexpression of *Ace2 gene* and higher activity of the detoxification enzymes esterases and partly MFO.

1. Introduction

The cowpea aphid (*Aphis craccivora* Koch) is a major insect pest attacking leguminous crops in Egypt (El-Ghareeb et al., 2002). Aphids cause significant economic damage by sucking sap from leaves, pods and other aerial tissues or indirectly through transmission of major viruses (Laamari et al., 2008). Aphid management strategy heavily relies on the use of synthetic insecticides such as organophosphates, carbamates, pyrethroids, and neonicotinoids (Jackai and Daoust, 1986; Shetlar, 2001; Tang et al., 2013). The extensive and repeated use of such insecticides has resulted in the development of resistance (Hollingsworth et al., 1994; Han and Li, 2004).

Pirimicarb, a carbamate insecticide, is recommended for cowpea aphid control (Egyptian Agricultural Pesticides Committee, 2016). However, its efficacy and sustainability could be threatened due to development of resistance. Target site based resistance, as an insensitive form of AChE, has been shown to be a major mechanism of primicarb resistance in green peach aphid *Myzus persicae* (Sulzer) (Moores et al., 1996). Acetylcholinesterase (AChE) is a target enzyme for organophosphate and carbamate insecticides. AChE terminates nerve impulses by catalyzing the hydrolysis of the neurotransmitter acetylcholine. In addition, non-target site resistance conferred by detoxification enzymes (enhanced metabolism) can occur (Li et al., 2007). Carbamates are supposed to be detoxified by esterases, glutathione S-transferases (GST) and cytochrome P450 oxidases (Cyt-P450) (Rufingier et al., 1999). The identification of the role of these detoxification enzymes can be deduced with synergists and biochemical determination.

The present study was conducted to investigate the potential mechanism of resistance to pirimicarb in the cowpea aphid using

* Corresponding author.
E-mail address: isabdalla@ucdavis.edu (I.S. Abdallah).

http://dx.doi.org/10.1016/j.cropro.2016.12.020
0261-2194/

bioassays, biochemical and molecular techniques. On the biochemical level, non-target site based resistance due to enhanced pirimicarb metabolism was studied. On the molecular level, resistance mechanism to pirimicarb was examined through the alteration of the AChE *gene* expression.

2. Materials and methods

2.1. Chemicals and insecticides used

Both technical grade (pirimicarb 99%) and commercial formulation (Aphox 50% DG) were used in this study. Technical grade was used for biochemical studies, while the commercial insecticide was used for bioassays. Acetylthiocholine iodide (ATChI), triton X-100, fast blue RR salt, piperonyl butoxide (PBO), S, S, S-tributyl phosphorotrithioate (DEF), glutathione (GSH), p-nitroanisole (p-NA), 1-chloro-2,4-dinitrobenzene (CDNB) and ethidium bromide (EB) were obtained from Sigma–Aldrich. 5, 5-dithio-bis (2-nitrobenzoic acid) (DTNB) was obtained from Roth, diethyl maleate (DEM) was obtained from Alfa-Aesar, reduced nicotinamide adenine dinucleotide phosphate (NADPH) was obtained from Sorachim, α-naphthyl acetate (α-NA) was obtained from Mpbio, DNA Marker GeneRuler 100 bp was obtained from Thermo Scientific.

Insecticides in this study used for selection, bioassay and synergism experiments were commercial formulations (Table 1). The insecticide groups included carbamates, organophosphates and neonicotinoids.

2.2. Test insects

Two cowpea aphid strains were used in this study. The susceptible S strain was obtained from the Plant Protection Research Institute, Agricultural Research Center, Egypt-in June 2007. This strain was used as a reference in resistance comparisons and in biochemical and molecular assays. This strain was reared for more than seven years in the laboratory without prior exposure to any insecticide and proved to be susceptible (Kandil et al., 2013). The pirimicarb resistant (R) field strain was originally collected from a faba bean (*Vicia faba* L.) field located in Sharkia Governorate, Egypt. The strain was continuously selected with pirimicarb for 12 generations (Table 2). At the 12th generation, pirimicarb resistance stabilized. Both S and R strains were reared in the laboratory at 20 ± 3 °C with a photoperiod of 16:8 h (L: D). Aphids were reared on metallic stands in chambers and the insects were kept on faba bean seedlings grown in plastic pots (15 cm diameter). The pots with faba bean seedlings were maintained in another chamber without exposure to insecticides until needed.

3. Bioassay and cross resistance study

The toxicity of pirimicarb to the cowpea aphid was determined for both resistant and laboratory susceptible strains using leaf dipping technique as described by Moores et al. (1996). Commercial formulation of pirimicarb (aphox 50% DG) at the recommended field rate of 25 g AI/100 L H_2O was used. A preliminary dose-response study using serial concentrations of commercial pirimicarb showed that the rate of mortality was much higher in the laboratory susceptible strain compared to the resistant one. The concentrations used were 0.125, 0.180, 0.25, 0.375, 0.5, 0.75, 1, 1.5, and 3 ppm. Fresh faba bean leaves were dipped into pirimicarb solutions for 10 s, air-dried, then placed upside down on an agar bed in labeled Petri dishes (60 mm diameter). Ten apterous adults of *A. craccivora* were placed on treated leaf surface, while leaves dipped in water only served as controls. Five replicate batches of aphids were used. Mortality was recorded after 24 h as adults failing to coordinate forward movement when probed with a soft camel hair brush. Mortality was corrected by Abbott's formula (Abbott, 1925). The LC_{50} values were calculated by probit analysis using Ldp line (Ehabsoft V.1.0 software). The resistance ratio (RR) was calculated as LC_{50} of R strain/LC_{50} of S strain (Prabhaker et al., 1998). Cross resistance was determined by comparing the ratio of LC_{50} pirimicarb resistant strain divided by the LC_{50} of susceptible strain for all the tested insecticides. The aforementioned leaf dipping method was also used for cross resistance studies.

Table 2
Development of resistance in the laboratory *A. craccivora* strain selected with pirimicarb.

Generation	Slope ± SE	LC50 (mg L^{-1}) 95%CL	RR (fold)
Susceptible strain	1.639 ± 0.204	0.027 (0.025–0.038)	–
Parent strain	1.251 ± 0.204	0.459 (0.34–0.606)	17
2nd generation	1.451 ± 0.217	0.594 (0.468–0.797)	22
4th generation	1.728 ± 0.155	0.68 (0.578–0.802)	25.185
6th generation	1.561 ± 0.203	0.819 (0.686–1.008)	30.33
8th generation	1.525 ± 0.188	0.871 (0.713–1.1)	31.25
10th generation	1.548 ± 0.152	1.138 (0.95–1.393)	42.148
12th generation	1.591 ± 0.153	1.282 (1.071–1.579)	47.481

Resistance ratio (RR) = LC_{50} of selected generation/LC_{50} of susceptible strain.
CL: Confidence limit.

3.1. Synergism bioassay

The role of the detoxifying enzymes in conferring resistance to pirimicarb was investigated using the synergists DEF as esterase inhibitor, DEM as GST inhibitor and PBO as MFO inhibitor. Preliminary studies were conducted to determine the maximum concentration of the tested synergists that gave no mortality when used alone. Preliminary studies were conducted in order to select the proper concentration in a wide range of concentrations for each synergist that showed no mortality. The following concentrations of each synergist were tested; 5, 10, 12.5, 25, 50, 80 and 100 mg L^{-1}.

The maximum concentration of the synergist that gave no mortality to the susceptible strain was shown to be 10 mg L^{-1}. In order to standardize the bioassays for all synergists used, we used only one representative concentration that exhibited similar response against tested insects. This synergist concentration was

Table 1
The tested insecticides.

Common name	Trade name	Chemical class	Manufacturer
Pirimicarb	Aphox 50% DG	Carbamate	Syngenta
Carbosulfan	Marshel 25% WP	Carbamate	FMC
Fenirothion	Sumithion 50% EC	Organophosphorus	Sumitomo
Chlorpyriphos- methyl	Reldan 50% EC	Organophosphorus	Dow Agro Sciences
Malathion	Malson 57% EC	Organophosphorus	Ficom Organics
Thiamethoxam	Actra 25%WG	Neonicotinoids	Syngenta
Acetamiprid	Mospilan 20%SP	Neonicotinoids	Nippon Soda