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Effect of chlorfenapyr on cypermethrin-resistant *Culex pipiens pallens* Coq mosquitoes

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ABSTRACT

Chlorfenapyr is a promising pyrrole insecticide with a unique mechanism of action that does not confer cross-resistance to neurotoxic insecticides. The effect of chlorfenapyr on pyrethorid-resistant *Culex pipiens pallens* Coq (Diptera: Culicidae) has not been fully investigated under laboratory conditions. In this study, cypermethrin-resistant *C. p. pallens* exhibited 376.79-fold and 395.40-fold increase in resistance to cypermethrin compared with susceptible strains after exposure for 24 and 48 h, respectively. Larvae and adults were tested for susceptibility using dipping, topical, and impregnated paper methods as recommended by the WHO. No cross-resistance to chlorfenapyr was found. Increased mortality was apparent between 48 and 72 h, indicating a slow rate of toxic activity. Synergism experiments with piperonyl butoxide (PBO) showed an antagonistic effect on chlorfenapyr toxicity. Mixtures of chlorfenapyr and cypermethrin could therefore provide additional benefits over either insecticide used alone. Mixtures of 5 ng/ml chlorfenapyr and 500 ng/ml cypermethrin exhibited a slight synergistic effect on cypermethrin-resistant mosquitoes (3.33, 6.84 and 2.34% after 24, 48 and 72 h exposure, respectively. This activity was lost when the chlorfenapyr concentration was increased to 10 or 20 ng/ml. Chlorfenapyr showed quite good results for pyrethroid-resistant *C. p. pallens*, and could improve public health by reducing the occurrence of mosquito bites and subsequently protecting against transmission of lymphatic filariasis and Japanese encephalitis.

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1. Introduction

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More than 300 mosquito species have been identified in China including *Culex pipiens pallens*, a major nuisance and vector of the human diseases lymphatic filariasis and Japanese encephalitis in northern and central China (Cui et al., 2006; Li et al., 2002). Aerosols, mosquito coils, electric mosquito chips and liquids, and insecticide-treated mosquito nets (ITNs) are widely used for controlling adult mosquitoes indoors. Pyrethroid insecticides have been used widely in agriculture and medicine in China since the 1980s due to their high insecticidal potency, fast knockdown and relatively low mammalian toxicity (Cui et al., 2006). Within the sphere of public health, most of the insecticidal ingredients in sanitation products are pyrethroids, including deltamethrin, cypermethrin, D-allethrin, prallethrin, imiprothrin, transfluthrin, and meperfluthrin. The extensive use of pyrethroids has led to significant resistance in *C. p. pallens* across China (Gong et al., 2001; Liu et al., 2009, 2013; Wang et al., 2002). Two major mechanisms are believed to be responsible for insecticide resistance, namely the targeting of alternate sites (Chen et al., 2010; Liu et al., 2012; Song et al., 2007) and the increased activity of detoxification enzymes such as P450 monooxygenases (P450s) (Chen, 1992; Li et al., 2001; Zhu et al., 1998). Novel insecticides for controlling disease vectors are urgently required. Numerous researchers have reported that chlorfenapyr is a promising pyrrole insecticide with a unique mechanism of action that does not confer cross-resistance to neurotoxic insecticides. It is a pro-insecticide that is activated by P450s to the more active metabolite AC303268 that acts by uncoupling oxidative phosphorylation in mitochondria (Black et al., 1994). At present, chlorfenapyr that has been registered and used for controlling agricultural pests has not been registered for controlling mosquitoes in China. This compound exhibited negative cross-resistance in housefly (Scott et al., 2004), horn fly (Sheppard and Joyce, 1998) and tobacco budworm (Pimprale et al., 1997), no cross-resistance whatsoever in *Anopheles* (N'Guessan et al., 2007, 2009; Oliver et al., 2010; Pridgeon et al., 2008; Raghavendra et al., 2011b), *Aedes* (Paul et al.,

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2006), *Culex* (N'Guessan et al., 2009; Oxborough et al., 2010) or bed bug (Romero et al., 2010), and has been recommended by the WHO for use in public health only due to a slight toxicity to humans (WHO toxicological classification III). To date, the efficacy of chlorfenapyr against pyrethorid-resistant *C. p. pallens* has not been fully investigated under laboratory conditions. In this study, we assessed the efficacy of chlorfenapyr against cypermethrin-resistant *C. p. pallens* and consider the evidence for possible use in the field.

2. Materials and methods

2.1. Mosquito strains

Two laboratory *C. p. pallens* strains were used; (1) a susceptible strain (SS) that has been maintained in the laboratory for 50 years without exposure to any insecticides. (2) a cypermethrin-resistant (CR) strain that was subjected to heavy selection pressure (LC_{60-70}) for cypermethrin for over 3 years.

2.2. Larval bioassays

Larval bioassays were performed using the dipping method as described (Tao et al., 2006) with slight modifications Briefly, 1 ml insecticide solution of varying concentrations (or 1 ml acetone as control) was added to 199 ml distilled water containing 30 early fourth instar mosquito larvae. P450-mediated detoxification of cypermethrin was measured as described (Paul et al., 2006). The maximum non-lethal concentration of the P450 inhibitor piperonyl butoxide (PBO, 0.01%) was determined through preliminary experiment, and 1 ml PBO was add to 199 ml distilled water containing 1 ml insecticide solution and 30 early fourth instar mosquito larvae (or 1 ml acetone and 1 ml PBO as control).

2.3. Adult bioassays by topical method

Adult bioassays were performed using the topical method as described (Pridgeon et al., 2008) with slight modifications. A droplet (0.5 μl) containing six different concentrations of insecticide (or 0.5 μl acetone as control) was applied to the dorsal thorax of 30 3–4-day-old non-blood-fed female mosquitoes using a mircoapplicator (Burkard, UK), which were then anesthetized for 35 s using ether. In order to evaluate the detoxification activity of the P450 enzymes, the maximum non-lethal concentration of PBO (0.1%) was determined through preliminary experiment, and varying concentrations of chlorfenapyr containing 0.1% PBO were assayed. After treatment, adults were held in a 15 cm × 15 cm × 15 cm screened cage and provided with 10% sucrose solution.

2.4. Adult bioassays by impregnated paper method

Adult bioassays were performed using the impregnated paper method according to the standard contact exposure tube bioassay with insecticide impregnated paper procedure as described (WHO, 1998) with slight modifications. Test papers (Whatman No. 1) were treated with five different concentrations of chlorfenapyr dissolved in acetone (1.3 ml) and olive oil (0.7 ml), air dried in a fume hood for 24 h. Approximately 30 3–4-day-old non-blood-fed female mosquitoes were tested for 1 h at each exposure period. In order to evaluate the P450 detoxification enzyme activity, the maximum non-lethal concentration of PBO (10%) was determined through preliminary experiment. Bioassays were carried out by pre-exposure to the PBO-impregnated papers for 1 h, followed by exposure to chlorfenapyr-impregnated papers for 1 h, and then held in a 30 cm × 30 cm × 40 cm screened cage provided with 10% sucrose solution. Experimental mortality was recorded after 24 and 48 h for cypermethrin and 24, 48 and 72 h for chlorfenapyr.

Temperature and humidity were maintained at 26 ± 1 °C, 75 ± 10% RH throughout the bioassay period. All experiments were repeated in triplicate. Data are expressed as mean values ± SD of three independent experiments. All data analysis was performed using SPSS 13.0 software and the differences were considered as significant at $p \leq 0.05$ (ANOVA). Mortality was corrected using the Abbott's formula (Abbott, 1925). Synergism data from insecticide mixtures were analyzed as described previously (Bliss, 1939).

3. Results

3.1. Larval bioassays

Bioassay results on *C. p. pallens* larvae showed that cypermethrin-resistance was 376.79-fold and 395.40-fold higher in the CR strain than the SS strain after exposure to cypermethrin for 24 and 48 h, respectively (Table 1). Addition of PBO enhanced the toxicity of cypermethrin against the CR strain by 117.55-fold and 109.68-fold ($p \leq 0.05$) after exposure for 24 and 48 h, respectively, suggesting that the mechanism of resistance involved increased P450 detoxification activity.

The relative toxicity of chlorfenapyr against larvae of both the CR and SS strains was assessed, and no significant differences ($p > 0.05$) between strains was apparent; resistance ratios (RR) were 1.20-fold, 1.16-fold and 1.03-fold higher after exposure for 24, 48 and 72 h, respectively (Table 2), indicating that no cross-resistance to chlorfenapyr had occurred. Synergism experiments showed that PBO significantly antagonized chlorfenapyr toxicity with a synergism ratio (SR) of 0.90-fold, 0.79-fold and 0.85-fold (SS stain), and 0.98-fold, 0.83-fold and 0.82-fold (CR strain) after 24, 48 and 72 h, respectively ($p \leq 0.05$) (Table 2).

3.2. Adult bioassays by topical method

Chlorfenapyr toxicity was assayed against adult mosquitoes from both strains using the topical method. No significant difference was found in both strains ($p > 0.05$), and RR values were 1.23-fold, 1.29-fold and 1.37-fold at 24, 48 and 72 h after chlorfenapyr treatment, respectively (Table 3). Interestingly, synergism experiments showed a significant antagonistic effect ($p \leq 0.05$) of PBO on chlorfenapyr toxicity in adult mosquitoes of both strains; the SR values were 0.87-fold, 0.89-fold and 0.84-fold in the SS stain and 0.82-fold, 0.64-fold and 0.70-fold in the CR strain at 24, 48 and 72 h, respectively.

3.3. Adult bioassays by impregnated paper method

Chlorfenapyr toxicity towards adult insects was also evaluated in both strains using the impregnated paper method. No significant difference was found in both strains ($p > 0.05$), and the resistance ratio (RR) was 1.33-fold, 1.73-fold and 1.57-fold at 24, 48 and 72 h, respectively (Table 4), which indicated that the susceptibility to chlorfenapyr was similar in both strains. Chlorfenapyr could therefore be effective for controlling CR strain adult insects. Synergism experiments again showed a significant antagonistic effect ($p \leq 0.05$) of PBO on chlorfenapyr toxicity in both strains; SR values were 0.91-fold, 0.85-fold and 0.89-fold in the SS strain and 0.91-fold, 0.89-fold and 0.90-fold in the CR strain at 24, 48 and 72 h, respectively.

3.4. Insecticide mixing effects

The toxicity of mixtures of cypermethrin and chlorfenapyr against CR strain larvae was assessed using the dipping method, and the mortality rate of mosquitoes treated with a mixture of cypermethrin and chlorfenapyr was higher than treatment

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