Correlation of Pyrethroid Structure and Resistance Level in *Culex quinquefasciatus* Say from Saudi Arabia

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INTRODUCTION

Pyrethroids are one of the most widely used insecticides in the world. When photo-stable pyrethroids are introduced as agricultural insecticides, they are promising compounds because of their broad and potent insecticidal activity, and their low toxicity to mammals. However, extensive use of pyrethroids results in the development of resistance in many agriculturally and medically important pests.1-4 Early pyrethroids can not be used near aquatic environments because of their high fish toxicity. However, some pyrethroids with low fish toxicity have been developed for control of pests in rice paddy fields.5,6 A major concern is the development of resistance in non-target insects such as mosquitoes, including vectors of tropical diseases, through the use of these insecticides.7

A pyrethroid resistant strain of *Culex quinquefasciatus* Say (JPal-per strain), originally collected from Saudi Arabia, showed high resistance (1546-fold) to permethrin.8 Previously, we reported that the major mechanism of permethrin resistance in this strain is enhanced permethrin detoxification via cytochrome P450(s) in addition to a *kdr* (knock down resistance)-type nerve insensitivity.9 We also estimated the resistance levels of JPal-per strain against three organophosphates (fenitrothion, profenofos and parathion), a carbamate (carbaryl) and an insect growth regulator, pyriproxyfen. The resistance ratios to these insecticides were relatively low and ranged between 1.8 to 6.4-fold.9 However, the spectrum of resistance against pyrethroid insecticides in JPal-per strain is still unknown. In this study we investigated the toxicity of 13 pyrethroid insecticides against the JPal-per strain and discuss the correlation between their chemical structures and resistance levels.

MATERIALS AND METHODS

1. Insects
The pyrethroid resistant strain of *Culex quinquefasciatus* (JPal-per) obtained from Dr. J. Hemingway (Univ. of Wales Cardiff, UK) was collected from Palestine Street in the University district of Jeddah, Saudi Arabia and selected with permethrin for 20 consecutive generations at a mortality level of 60-75%.8 The susceptible strain was obtained from National Institute of Infectious Diseases collected from Chichijima, Ogasawara Island, Japan in 1968 and cultured without exposure to insecticides. Larvae were fed ground rat pellets. Adults were maintained on 10% sucrose and females were given blood meals from mice. Both strains were reared at 27±1°C and a photo period of 16:8 (L: D) hr.

2. Chemicals
The following insecticides were used: bifenthrin (90.4%; FMC), deltamethrin (99.9%; AgrEvo), etofenprox (96.0%; Mitsui Toatsu Chemicals, Inc.), and insecticides obtained from Sumitomo Chemical Co., Ltd. including allethrin (91.1%), cyfluthrin (88.4%), cypermethrin (94.5%), cyphenothrin (94.3%), fenvalerate (94.9%), furamethrin (88.0%), permethrin (91.2%), profenofos (94.0%), resmethrin (94.1%) and tetramethrin (94.3%).

3. Larval Bioassay
Larval bioassays were carried out using standard bioassay techniques for mosquito larvae.10 Twenty to thirty early fourth-instar larvae were exposed to different concentrations of pyrethroids in 50 ml of distilled water. The treated individuals were kept at 27±1°C and the mortality was assessed 24 hr after exposure. Ethanol was used as a carrier of the insecticides and control animals were dosed with ethanol only. The alcohol concentration never exceeded 1% of the total volume (sublethal concentration). At least 3 replicates were run for each insecticide concentration. LC50 values for each insecticide were calculated using log-probit mortality regression analysis.11 The resistance ratio for each insecticide was calculated by dividing the LC50 values of the resistant strain by that of the susceptible strain.

RESULTS AND DISCUSSION
The toxicity of 13 pyrethroids to susceptible and resistant JPal-per strains is listed in Table 1. We observed that the JPal-per strain showed cross-resistance to all pyrethroids tested. The resistance ratios, however, ranged from 5.6 to
Pyrethroids can be classified into two types according to the absence (type I) or presence (type II) of an α-cyano group in the alcohol moiety. JPa1-per strain showed higher resistance to permethrin, etofenprox, phenothrin and resmethrin which belong to type I pyrethroids. Furthermore, three of these insecticides (permethrin, etofenprox, phenothrin) have a 3-phenoxybenzyl moiety in their chemical structures (Group I, Table 2). Etofenprox is unique because it contains an ether bond instead of the more common ester bond between the acid- and alcohol-moieties found in pyrethrins and most synthetic pyrethroids. The ether bond of etofenprox, however, does not reduce but rather increase the high level of resistance found in other pyrethroids having a phenoxybenzyl group such as permethrin and phenothrin. On the other hand, JPa1-per strain showed only moderate resistance to pyrethroids that have a 3-phenoxybenzyl moiety with an α-cyano group (Group II, Table 2). The resistance ratios of JPal-per strain against these 4 compounds were between 39- to 56-fold. The only difference between permethrin and cypermethrin or phenothrin and cyphenothrin which belong to type I pyrethroids. Furthermore, three of these insecticides (permethrin, etofenprox, phenothrin) have a 3-phenoxybenzyl moiety in their chemical structures (Group I, Table 2). Etofenprox is unique because it contains an ether bond instead of the more common ester bond between the acid- and alcohol-moieties found in pyrethrins and most synthetic pyrethroids. The ether bond of etofenprox, however, does not reduce but rather increase the high level of resistance found in other pyrethroids having a phenoxybenzyl group such as permethrin and phenothrin. On the other hand, JPa1-per strain showed only moderate resistance to pyrethroids that have a 3-phenoxybenzyl moiety with an α-cyano group (Group II, Table 2). The resistance ratios of JPal-per strain against these 4 compounds were between 39- to 56-fold. The only difference between permethrin and cypermethrin or phenothrin and cyphenothrin is the presence or absence of an α-cyano group, suggesting that the α-cyano group affects resistance mechanism(s) of the JPa1-per strain. In contrast, the JPa1-per strain showed relatively low levels of resistance to tetramethrin and allethrin (the resistance ratios were 5.6- and 8.6-fold, respectively), which have neither a 3-phenoxybenzyl moiety nor an α-cyano group (Table 2). The LD_{50} values to these two compounds were larger than most pyrethroids having a 3-phenoxybenzyl moiety with an α-cyano group (Table 1).

Another mechanism of pyrethroid resistance is detoxification by metabolic enzymes. Previously, we investigated the synergistic effects of oxidase inhibitors on the toxicity of permethrin and in vitro metabolism of [14C]-permethrin, and concluded that cytochrome P450 monoxygenases are one of the major mechanisms responsible for permethrin resistance in the JPa1-per strain. In this metabolism study, we observed that monoxygenases hydroxylated...
the phenoxybenzyl moiety of permethrin. 9)

Cytochrome P450s play a very important role in resistance to various insecticides in many pest insects. 8)9) In the pyrethroid resistant strain of the house fly (LPR), enhanced levels of pyrethroid detoxification via a cytochrome P450 (CYP6D1) are related to resistance. 20)21) Curiously, the LPR strain shows extremely high resistance to pyrethroids with or without an $\alpha$-cyano group though the presence of phenoxybenzyl moiety is always associated with high levels (>900-fold) of resistance. 22) This difference may come from a large variation in substrate specificity of different P450s which is one of the remarkable features of the monooxygenases. For example, cytochrome P450 1A1 (CYP1A1) can metabolize more than 20 substrates while CYP7A1 has only one known substrate. 23) In the JPa1-per strain, the P450 that is involved in resistance may have higher substrate specificity than CYP6D1 (i.e., the isoform that hydroxylates only the 3-phenoxybenzyl moiety (without an $\alpha$-cyano group) may cause the resistance). Recently, we cloned and determined the nucleotide sequence of cytochrome P450 cDNAs (CYP6E1 and CYP6F1) from JPa1-per strain in addition to four other partial cDNAs. 24, 25) CYP6F1 was observed to be over-expressed in the JPa1-per strain relative to the susceptible strain suggesting the possible involvement of this isoform in insecticide resistance. 26) Heterologous expression and characterization of these isoforms individually will bring us closer to understanding the resistance mechanisms of the JPa1-per strain.

Sheppard (1995) selected horn flies (Haematobia irritans) with a cyanopyrethroid, cyhalothrin. The resultant strain showed high resistance to all cyanopyrethroids tested (671- to 12,831-fold) as compared to non-cyano group pesticides (30- to 69-fold). By means of a PBO synergism study, it was concluded that enhanced activity of oxidative enzyme(s) is the major mechanism of cyanopyrethroid resistance in this strain. 26) It seems that oxidative enzyme(s) recognizes the $\alpha$-cyano group in the substrates, and that different types of cytochrome P450(s) can be selected depending on the presence or absence of an $\alpha$-cyano group in the pyrethroid insecticide used for the selections.
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