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INSECTICIDE RESISTANCE AND RESISTANCE MECHANISMS IN *CIMEX HEMIPTERUS* (HEMIPTERA: CIMICIDAE) AND THEIR SUSCEPTIBILITY TO PYRETHROID-NEONICOTINOID MIXTURES

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Abstract The DDT, deltamethrin, and malathion susceptibilities of five tropical bed bug (*Cimex hemipterus*) strains, and their resistance mechanisms were investigated using synergism studies and detection of *kdr* mutations. All *C. hemipterus* strains showed high resistance to DDT and deltamethrin and moderate to high resistance to malathion. Synergism studies revealed that P450s conferred resistance to DDT and deltamethrin, while esterases conferred resistance to malathion. Molecular detections revealed three *kdr* mutations M918I, D953G, and L1014F with M918I + L1014F mutations possibly conferring *super-kdr* characteristics in *C. hemipterus*. The performance of two pyrethroid-neonicotinoid mixtures (Temprid SC and Tandem) treated on glass and filter paper also was evaluated. All strains displayed low to very high resistance to Temprid SC. In comparison, all strains displayed low resistance to Tandem. Temprid SC and Tandem residues on glass provided significantly faster knockdown and higher mortality than filter paper.

Key words synergism studies, metabolic resistance, *kdr*, tropical bed bugs

INTRODUCTION

The common bed bug *Cimex lectularius* L. and tropical bed bug *C. hemipterus* (F.) are cryptic, nocturnal ectoparasites adapted to blood-feeding on humans (Usinger, 1966). Both species have undergone a global resurgence over the last 25 years (Doggett et al., 2018). Insecticide resistance is considered as the most significant contributing factor to the resurgence (Romero et al., 2007). Three main mechanisms have been identified for the development of insecticide resistance; target site insensitivity (e.g., *kdr* mutations), increased metabolic detoxification (e.g., cytochrome P450 monooxygenases [P450s], esterases, and glutathione S-transferases [GSTs]), and penetration resistance (e.g., cuticular thickening and additional protein deposition in the cuticle) (Davies et al., 2012; Dang et al. 2017a).

Efforts have been undertaken to study bed bug resistance and their mechanisms, mainly concentrating on *C. lectularius* (Dang et al., 2017a). Knowledge of the resistance mechanisms in *C. hemipterus* remains limited. This study investigated underlying resistance mechanisms in tropical bed bugs from Australia and Malaysia. We evaluated five *C. hemipterus* strains' susceptibility to DDT, deltamethrin, and malathion. The potential involvement of metabolic resistance (P450s, esterases, and GSTs) was investigated using synergists, namely piperonyl butoxide (PBO), *S,S,S*-tributyl phosphotritioate (DEF), and diethyl maleate (DEM). *kdr* genotypes on the voltage-gated sodium channel (VGSC) gene were assessed (Dang et al., 2015). Finally, the performance of two pyrethroid-neonicotinoid mixtures (Temprid SC and Tandem) on two substrates (glass versus filter paper) against the *C. hemipterus* strains. was determined

MATERIALS AND METHODS

Five populations of *C. hemipterus* (Australia: Queensland [QLD-AU, established 2007], Malaysia: Kuala Lumpur [KL-MY, 2005], Christian [CH-MY, 2015], Green Lane [GL-MY, 2015], and Tanjung Tokong [TT-MY, 2015]) were used in the experiments. The strains were maintained in the Universiti Sains Malaysia, at 27±2 ºC and 75±10 % (RH), with a 12-hour photoperiod. We fed the bed bugs with freshly drawn rabbit blood using the Hemotek membrane feeding system (Discovery Workshops, Accrington, UK). As an insecticide-susceptible *C. hemipterus* strain was not available worldwide (Dang et al., 2015; 2021), the susceptible Monheim *C. lectularius* strain was used as the control. None of the strains underwent insecticide selection. All bed bug species were confirmed using Usinger (1966).

Individual DNA of the TT-MY (n=7), CH-MY(n=5), and GL-MY(n=5) strains was extracted, purified, and analyzed for *kdr*-related genes by PCR amplification, based on procedures described in Dang et al. (2015) and Soh and Veera Singham (2021).

To detect insecticide resistance, discriminating doses of deltamethrin (191 mg AI m^{-2}), DDT (2%), and malathion (5%) were assayed by the surface contact method (Dang et al., 2017b; 2021). In the synergism assays, each bed bug was pre-treated topically with 1 µl solution of PBO (50 µg/µl), DEF (15 µg/µl), or DEM (50 µg/µl) dissolved in acetone, 2 h before insecticide exposure (Dang et al., 2021). Knockdown was recorded at regular time intervals for up to 72 h (deltamethrin and malathion) and 120 h (DDT). In the trials of the insecticide formulations, filter paper (90 mm diam. Whatman grade 1) and glass (90 mm diam. × 15 mm height glass Petri dish) were treated with 1 ml of Temprid SC (label application rate: 0.075 %, equating to 0.05% imidacloprid and 0.025% beta-cyfluthrin) or Tandem (0.13 %, equating to 0.1% thiamethoxam and 0.03% lambda-cyhalothrin) diluted in water, and then left to air-dry overnight. Knockdown was recorded at selected time intervals for up to 120 h. Mortality was recorded daily up to 120 h. Three replicates of ten mixed adult bed bugs were done for each insecticide and each strain in each experiment.

Knockdown times (KTs) for 50% and 95% of the bed bugs (KT_{50}s and KT_{95}s) were generated by probit analysis using GraphPad Prism 5.00 (GraphPad Software, San Diego, California USA). Statistical significance was examined via one-way ANOVA with Tukey HSD test using GraphPad Prism. Resistance ratios at KT_{50} (RR_{50}) were calculated by dividing KT_{50} of the resistant strain by KT_{50} of the Monheim strain.

RESULTS AND DISCUSSION

All five *C. hemipterus* strains displayed high levels of resistance to DDT (RR_{50}s >29-fold) and deltamethrin (RR_{50}s >224-fold). Synergism studies revealed that pre-treatment with PBO (inhibitor of P450s) significantly ($P<0.05$) suppressed resistance to deltamethrin in all five strains, and DDT in four strains (excluding the KL-MY strain). The addition of PBO also increased the susceptibility of the QLD-AU strain to malathion. P450s can metabolize a broad range of substrates and may lead to cross-resistance with different classes of insecticides (Mamidala et al., 2012; David et al., 2013). Thus, P450s presumably confer metabolic resistance to deltamethrin and other classes of insecticides (e.g., DDT, malathion) in *C. hemipterus*. Conversely, the pre-treatment of DEM (inhibitor of GSTs) did not effectively increase the susceptibility of all *C. hemipterus* strains to deltamethrin and DDT. DEM only suppressed malathion resistance in the QLD-AU strain. Apart from GSTs mediated resistance, other forms of resistance such as metabolic (e.g., P450s) and *kdr* could instead contribute towards deltamethrin and DDT resistance in the five strains.

Molecular analyses identified three putative *kdr* mutations, M918I, D953G, and L1014F, in the CH-MY (n=5) and GL-MY (n=5) strains. The QLD-AU (M918I and L1014F) and KL-MY (L1014F) strains were previously reported by Dang et al. (2015). Bioassays and molecular analyses of the TT-MY strain (n=7) showed that individuals (5/7) that were knocked down during the first hour by deltamethrin had either D953G+L1014F (homozygous susceptible: M918, 1/5) or M918I+D953G+L1014F (heterozygous resistance-susceptible: I918, 4/5) mutations. Individuals (2/7) that were knocked down by deltamethrin after 12 h exposure displayed M918I+D953G+L1014F (homozygous resistance: I918) mutations (both D953G and L1014F mutations were homozygous resistance in all the seven individual insects). Taken together, the

results suggested M918I + L1014F mutations confer *super-kdr* resistance, which enhances deltamethrin resistance in *C. hemipterus* (Dang et al., 2015; Zhao et al., 2020; Soh and Veera Singham, 2021).

The five strains also showed moderate to high levels of resistance to malathion (RR_{50}s: 14.3 to >96.6-fold). The pre-treatment of DEF (inhibitor of esterases) effectively ($P<0.05$) reduced malathion resistance in all five strains, suggesting that esterases are typically conferring resistance to malathion in *C. hemipterus*. However, altered acetylcholinesterases (AChEs) (e.g., F348Y mutation) may also be present against malathion in the *C. hemipterus* strains (Komagata et al., 2021). Further studies are warranted.

In the insecticide formulation residual trials, the five strains had varying degrees of resistance to Temprid SC on glass; TT-MY (6.5-fold, low resistance), QLD-AU, and GL-MY (12.8–21.6-fold, moderate resistance), KL-MY (48.2–49-fold, high resistance), CH-MY (128.2-fold, very high resistance). The pre-existing metabolic resistance mechanisms (e.g., P450s) found above may be conferring cross-resistance to the imidacloprid in Temprid SC. In comparison, all *C. hemipterus* strains displayed low resistance to Tandem (1.8–8.3-fold). Tandem produced faster mortality than Temprid SC in all strains. One possible factor for the findings is that the neonicotinoid concentration in Tandem is almost twice that of Temprid SC at the label application rate (Wang et al., 2015). In addition, Temprid SC and Tandem residues on glass provided significantly faster knockdown and higher mortality than on filter paper. Despite the CH-MY strain (43.3%), Temprid SC residues on glass caused high mortality to the KL-MY (63.3 %), GL-MY (70%), TT-MY (100%), and QLD-AU (100%) after 120 h exposure. In comparison, Tandem residues caused predominantly high mortality (100%) to all five strains. However, both residues on the filter paper caused low mortality (<30%) to all the strains after 120 h exposure. Compared with a non-porous surface (glass), the absorption of an insecticide on a porous surface (filter paper) would be higher, resulting in less insecticide being available on the surface to bed bugs. Importantly, bed bugs typically avoid smooth surfaces and prefer rough, porous substrates (Wang et al., 2016). Based on the findings, for *C. hemipterus* management, insecticide formulations incorporated with PBO and with a high concentration of active (e.g., neonicotinoid) at label application rate should be preferentially used. Ideally, rotation of insecticides with different modes of action (e.g., essential oil-based products, diatomaceous earth) and non-chemical methods (e.g., vacuums and extremes in temperature) should be incorporated in the best management practices.

CONCLUSION

The present study showed that multiple resistance mechanisms (e.g., P450s and esterases mediated metabolic resistance, *kdr* resistance) are involved in resisting different insecticides in *C. hemipterus*. Furthermore, M918I+L1014F mutations most likely confer *super-kdr* characteristics against deltamethrin and DDT in *C. hemipterus*. Although Temprid SC and Tandem residues on glass provided high residual efficacy to *C. hemipterus* strains, their residues on filter paper provided very low residual efficacy to all strains. Taken together, integrated pest management (IPM) strategies, including the rotation of insecticides with different modes of action and the use of non-insecticidal control methods, should be considered in the management of *C. hemipterus* infestations.

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