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Resistance and detoxification enzyme activities to bifenthrin in *Oxycarenus hyalinipennis* (Hemiptera: Lygaeidae)

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ABSTRACT

Bifenthrin, a neurotoxic insecticide, is used to control sucking and chewing insect pests. This study reports the development of resistance in a field collected *Oxycarenus hyalinipennis* population treated in the laboratory with bifenthrin for ten successive generations, which developed 163.0 and 781.4-fold resistance to bifenthrin as compared to a susceptible laboratory and the parental field population, correspondingly. The bifenthrin resistant strain displayed a high cross resistance to lambda cyhalothrin (resistance ratio RR = 399.8), while, no cross resistance between bifenthrin and profenofos, chlorpyrifos, imidacloprid or chlorfenapyr was recorded. The results showed bifenthrin resistance in *O. hyalinipennis* to be unstable with high reduction in lethal concentration (LC_{50}) ranging from 531.38 to 65.0 mg/L (in contrast to the susceptible strain) when reared for up to five generations without application of bifenthrin. The current research showed that activities of glutathione S-transferase, acid and alkaline phosphatases increased up to the 10th generation as compared to five generations (G_{11}-G_{15}) when selection pressure was removed, while the activities of total esterase and acetylcholinesterase were less than glutathione S-transferase, acid and alkaline phosphatases. It appeared that increased metabolism facilitated by the detoxification enzymes was a major cause for bifenthrin resistance in *O. hyalinipennis*.

1. Introduction

Cotton (*Gossypium hirsutum* L.) is the most important crop, used for vegetable oil and fiber as well as it is the source of foreign exchange in Pakistan (Akram et al., 2013), while average yield per acre of cotton is less than in other cotton producing countries (Bakhsh et al., 2005). Among various factors, insect pests are the major cause of low cotton production (Kannan et al., 2004). It is reported that 93 to 145 chewing as well as sucking insect pest species are involved in cotton yield losses (Smith and Brambila, 2008). Among sucking insects, dusky cotton bug, *Oxycarenus hyalinipennis* Costa (Hemiptera: Lygaeidae) is a pest of many economic crops including cotton and okra (El-Rahim et al., 2015). Nymphs and adults of *O. hyalinipennis* get nutrition and water from seeds and leaves of various plants and exert quantitative and qualitative losses to cotton (Khan and Ahmed, 2000). Application of different insecticides has been reduced for bollworms after the introduction of Bt cotton (Hofs et al., 2004), but Bt cotton was unable to provide resistance against several sucking insects i.e., the incidence of dusky cotton bug rose in Bt cotton as compared to non-Bt cotton (Patil and Rajanikanth, 2005). *O. hyalinipennis* has attained resistance to various insecticides belonging to different groups (Ullah et al., 2016a, 2016b; 2016c). In Pakistan continuous application of different insecticides has led to resistance development in *O. hyalinipennis* against various insecticides belonging to different groups (Khan et al., 2014). The knowledge on resistance mechanism in insects is used for developing successful management programs for combating resistance.

In addition to significant improvements in technology, merely restricted control measures have been developed to manage the population of *O. hyalinipennis*. It is reported that about 90% of farmers use chemicals sprays to protect their crops (Prayogo and Tengkano, 2005) and the continuous use of insecticides against a specific insect over a long period of time causes resistance. Synthetic pyrethroids are considered as agricultural pesticides showing high toxicity against several insects including resistant strains and low toxicity to birds, mammals and rapid biodegradability. However, they are extremely toxic to aquatic organisms including fish. These are considered as a group of neurotoxic insecticides causing paralysis by targeting the insect nervous system (Ali, 2012). In insects, numerous mechanisms facilitate resistance to pyrethroid, organochloride, organophosphate and carbamate insecticides. Resistance mechanisms i.e., altered target site insensitivity, reduced penetration, and metabolic resistance mediated by detoxifying enzymes such as glutathione transferases (GST), esterases and acetylcholinesterase (AChE) assist to develop resistance against insecticides (Ahmad and McCaffery, 1999).

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Bifenthrin and other insecticides are being used in the field for the management of *O. hyalinipennis*. Hence, in the current study, the potential of *O. hyalinipennis* to attain resistance against bifenthrin was examined through selection pressure in the laboratory and the mechanism of resistance was also studied by checking the activities of detoxification enzymes. Moreover, the cross resistance of bifenthrin in *O. hyalinipennis* to different insecticides was also determined.

2. Material and methods

2.1. Insect collection and rearing

Two populations of *O. hyalinipennis* were collected from two different places by following the resistance selection method used by Rehan and Freed (2014). One population of *O. hyalinipennis* was randomly collected by plucking infested bolls from cotton fields in Multan, in order to generate bifenthrin resistant population, while another population of *O. hyalinipennis* was also collected from a cotton field of Bahauddin Zakariya (BZ) University, Multan, reared in Lab. which was designated to be a susceptible Lab-strain. The adults and nymphs were separately placed in plastic jars ($15 \times 6 \times 6\ cm^3$) by using an aspirator. Fresh China rose, *Hibiscus rosasinensis* Linnaeus twigs along with opened *G. hirsutum* bolls, were used for rearing of both populations in same sized jars as mentioned above at 27 ± 2 °C and 65 ± 5% (RH) with a 14 light: 10 dark hour photoperiod. Every 48 h leaves were replaced and linted cotton seeds were placed in Petri dishes (5×5 cm) for egg laying (Ullah et al., 2016a).

2.2. Insecticides

Different insecticides which are generally used in the cotton field were selected to conduct experiments (Table 1).

2.3. Generating susceptible (Lab-strain) and bifenthrin resistant (BR) population of O. hyalinipennis

The collected insects from Multan were divided into two sets. One was used for generating a bifenthrin resistant (BR) strain by exposure to bifenthrin for up to 10 consecutive generations, while the second was reared without any insecticidal applications. Bifenthrin at different concentrations (mg/L) of 3, 6, 13, 26, 52, 104, 208, 417, 834 and 1669 were used for selection of *O. hyalinipennis* from 1st generation (G_1) to the 10th generation (G_{10}), respectively. The lowest dose of bifenthrin (3 mg/L) showed 50% mortality of the test population, therefore this dose was used. Approximately 1000 adults were used for selection of *O. hyalinipennis* with bifenthrin. At the 10th generation, a resistant population was developed which was designated to be the bifenthrin-resistant (BR) strain, whereas the second group was reared along with the BR strain without using any insecticide, designated to be the unselected strain (UNSEL). The population collected from B.Z. University was named as reference (Lab-strain) which was also reared up to the 15th generation without any selection pressure (Rehan and Freed, 2014).

2.4. Bioassay method

The bioassays were conducted by using different insecticides and 200 adults of *O. hyalinipennis* were used in each treatment including the control, while each treatment (5 levels) was repeated four times for determining the lethal concentration (LC_{50}) of each insecticide. Toxicity was evaluated using the leaf dip method by using China rose leaves on *O. hyalinipennis* adults (Afzal et al., 2015a; Ullah et al., 2016b). The leaves were dried for 2 h at room temperature after dipping for 50 s in the test solution. The treated leaves were placed in 5 cm diameter Petri dishes containing moist filter papers to prevent leaves from each drying. The mortality data was recorded after 48 h for conventional insecticides, and for 72 h in the case of new chemistry insecticides.

2.5. Evaluation of cross resistance with bifenthrin

The possible cross resistance of bifenthrin in *O. hyalinipennis* to both new chemistry and conventional insecticides was determined by bioassay methods before and after selection with bifenthrin.

2.6. Stability of bifenthrin resistance in selected and unselected O. hyalinipennis

Stability of bifenthrin resistance in *O. hyalinipennis* was checked after rearing the bifenthrin resistant (BR) and unselected strain for five generations 10th generation (G_{10}) to 15th generation (G_{15}) without any selection pressure. At 11th and 15th generations, bioassays were conducted as described previously.

2.7. Enzyme preparation

Samples for enzymatic study of each generation of bifenthrin resistant (BR) and susceptible Lab-strain were prepared by following the methodology of Tian et al. (2014) with little modification. For sample preparation, three adults of *O. hyalinipennis* at each generation (G_1 to G_{15}) were crushed in an Eppendorf tube containing 150 µl 0.15 M NaCl. A final volume of 500 µl was attained by adding more NaCl after the

Table 1
List of insecticides used in the experiment.

Insecticide (Active Substance)	Trade name	Formulation	IRAC MoA classifications	Group
Bifenthrin	Talstar®, FMC	10 EC	Sodium channel modulators (Nerve action)	Synthetic pyrethroid
Lambda cyhalothrin	Karate®, Syngenta	2.5 EC	Sodium channel modulators (Nerve action)	Synthetic pyrethroid
Profenofos	Curacuron®, Syngenta	50 EC	Acetylcholinesterase (AChE) inhibitors (Nerve action)	Organophosphate
Imidacloprid	Confidor®, Bayer	20SL	Nicotinic acetylcholine receptor (nAChR) agonists	Neonicotinoid
Chlorpyrifos	Lorsban®, Arysta	40 EC	Acetylcholinesterase (AChE) inhibitors (Nerve action)	Organophosphate
Chlorfenapyr	Saquadron®, FMC	360SC	Uncouplers of oxidative phosphorylation	Pyrrole