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Selection of bifenthrin resistance in cotton mealybug *Phenacoccus solenopsis* Tinsley (Homoptera: Pseudococcidae): Cross-resistance, realized heritability and possible resistance mechanism

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ABSTRACT

Cotton mealybug *Phenacoccus solenopsis* Tinsley is an important pest of cotton in Pakistan, and its management is difficult due to the development of insecticide resistance. This research was conducted to characterize the bifenthrin resistance in populations of *P. solenopsis* and different parameters such as cross-resistance, realized heritability and possible resistance mechanisms were studied to improve the management of this important pest. A field-collected population was selected with bifenthrin in the laboratory for 14 generations and developed a resistance of 178-fold. The realized heritability of bifenthrin resistance was 0.54 in the selected population. The toxicity of bifenthrin was synergized by the addition of either piperonylbutoxide (PBO) or S,S,S tributylphosphorotrithioate (DEF) which suggests a general metabolic resistance due to possible involvement of mono-oxygenases or esterases. However, the resistant population did not develop a significant cross-resistance to either buprofezin, chlorpyrifos or lambda-cyhalothrin. These data suggest that alternative insecticide-based management programs can be developed for this pest in the short-term, but resistance management strategies which can reduce the sole reliance on insecticides are still needed.

1. Introduction

Insecticides are commonly used to improve agricultural productivity in developing countries (Karunamoorthi et al., 2012), and are known as effective tools for numerous pests of agricultural importance (Sayyed and Crickmore, 2007; Ishtiaq and Saleem, 2011; Afzal et al., 2015a). Insecticide use improves productivity by minimizing the potential of insect pests to create infestations, but also on the other hand increases production costs and can lead to the development of resistance in insect pest species (Metcalf, 1989; Siqueira et al., 2001). Insects build up resistance to insecticides through uninterrupted utilization as well as by using the natural phenomenon of cross-resistance that develops because of previous exposure to different insecticides (Basit et al., 2011, 2013). In heavily sprayed crops like cotton, a suitable resistance management plan is required having potential to fulfill the pest management demand while decreasing the selection pressure on special target sites within the insect (Young et al., 2003).

Multiple kinds of chewing and sucking pests attack the cotton crop during the season, creating damage to crop productivity and yield (Afzal et al., 2015a). *Phenacoccus solenopsis* Tinsley (Homoptera: Pseudococcidae) is an important polyphagous pest and has caused significant yield losses to cotton crops for growers in some regions of Pakistan and other countries in Asia and in the United State of America (Abbas et al., 2005; Mahmood et al., 2011; Kumashiro et al., 2001; Nagrare et al., 2009; Wang et al., 2009; Hodgson et al., 2008). More than 40% losses of the cotton crop have been reported from Pakistan during 2007 as a result of *P. solenopsis* infestation (Pakistan cotton statistics, 2007). Management of *P. solenopsis* in Pakistan and many other countries has

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relied on broad-spectrum synthetic pyrethroid insecticides (Saddiq et al., 2014). Bifenthrin is commonly recommended for sucking pests in cotton including *P. solenopsis* (Saeed et al., 2007). Farmers have applied this insecticide in cotton fields to suppress the *P. solenopsis* populations but excessive applications of bifenthrin eventually resulted in resistance development. Bifenthrin is an insecticide among synthetic pyrethroids having stomach as well as contact activity (Thomson, 1998). It mainly acts upon sodium channels of insect nervous systems, causing these channels to open for a long time, thus increasing cell permeability and ultimately causing the death of insects (Brown, 2005).

Some basic knowledge about the extent of the resistance and the potential for cross-resistance to other classes of insecticides is essential to develop insecticide resistance management strategies (Roush and Croft, 1986). Also, characterizing the genetic heritability and the potential mechanisms of insecticide resistance are important in order to develop alternative programs. These data can guide pest managers to develop programs that allow reversion of existing resistances and to select alternative tools that can act independently of the current resistance mechanisms. We studied resistance to bifenthrin in a field population of *P. solenopsis* after its selection in the laboratory. To understand the possible mechanism underlying bifenthrin resistance, the involvement of metabolic enzymes was evaluated by using synergists. According to authors' best knowledge; it is the first report of bifenthrin resistance in *P. solenopsis*.

2. Materials and methods

2.1. Insecticides and synergists

Four commercial insecticides including bifenthrin (Talstar 10 EC, FMC, Pakistan), buprofezin (Fuzin 25 WP, 4B Group, Pakistan), lambda-cyhalothrin (Karate 2.5 EC, Syngenta, Pakistan) and chlorpyrifos (Lorsban 40 EC, Arysta Life Sciences, Pakistan) were used in this study. Two synergists including an esterase specific inhibitor, S,S,S tributylphosphorotrithioate (DEF; Sigma Ltd, UK) and a cytochrome P-450 monooxygenase inhibitor, piperonylbutoxide (PBO; Sigma Ltd, Poole, UK) were used for the synergism tests.

2.2. Insect collection and rearing

About 500 nymphs and adults were collected from a cotton field in Multan District. This field had a known history of frequent insecticide applications using a number of compounds each season. The cotton fields in that area receive heavy applications of different insecticides mainly of organophosphates (e.g., profenofos, chlorpyrifos, methamidophos), pyrethroids (e.g., bifenthrin, deltamethrin, cypermethrin, lambda-cyhalothrin) and some new chemistry insecticides (e.g., acetamiprid, imidacloprid, buprofezin) for the control of different cotton pests including *P. solenopsis*. The field collected population was brought in the laboratory and insects were reared by maintaining standard laboratory conditions specifically at 16:8 h (L: D), 27 ± 1 °C and 65 ± 5% R.H. This population was designated as Field Pop. Fresh tender shoots and leaves of China rose, *Hisbiscus rosa-sinensis* Linneaus were used as food of *P. solenopsis* and food was renewed after every 2 days. Transparent plastic jars (24 × 10 cm) were used to rear the insect. An insecticide-susceptible strain (Lab-PK) of *P. solenopsis* was reared in the Pak Arab Biological Control Laboratory (Fatima Sugar Research and Development Center, Muzaffargarh) for more than three years after its collection from cotton field of the Central Cotton Research Institute, Multan Pakistan. The population which was selected from the field population after performing base-line bioassays was called Bifen-Sel.

2.3. Bioassays

Bioassays were performed on Field Pop, Bifen-Sel and Lab-PK. Aqueous solutions of insecticides were prepared and serially diluted. Four concentrations of each insecticide were used in each insecticide bioassay. Three-day-old second instar nymphs were used in the bioassays. Fresh leaves of *H. rosasinensis* were used for bioassay according to the Leaf-dip method (Ahmad et al., 2007; Afzal et al., 2015a). The leaves after dipping for 10 s in insecticide solution were placed in Petri dishes (5 cm in diameter) with moist filter papers (Afzal et al., 2015a) and insects were exposed to insecticide treated leaves. A total of 40 nymphs, 10 nymphs per replication, for each insecticide concentration were used. For control, 20 nymphs with water-treated leaves were kept in Petri dishes. Numbers of insects tested in single bioassay including control were 180. Mortality data of bioassays was obtained after 48 h of treatment with bifenthrin, lambda-cyhalothrin, and chlorpyrifos, while for buprofezin, mortality data was taken after 96 h exposure. The nymphs were touched individually by using camel hair brush to check mortality and non-moving nymphs were considered dead.

2.4. Selection

Nymphs were selected at every generation from G_1 to G_{14} with bifenthrin. The concentrations for selection were determined by performing bioassay of bifenthrin on Field Pop. This bioassay provided different lethal concentrations that were then used for selection. Leaf dip method with same protocol as mentioned above was used for selection of population. About 100 to 200 nymphs were selected per generation (Table 1). Mortality was judged 48 h after insecticide exposure.

2.5. Synergism analysis

PBO and DEF concentrations were tested to recognize the uppermost non-lethal dose. Acetone (analytical reagent; Fisher Scientific, Lough borough, UK) was mixed with PBO or DEF and mixed in serial solutions having insecticide concentrations. The nonlethal concentrations, 5 mg/ml PBO and 10 mg/ml DEF, were used for Bifen-Sel (G_{15}), while a concentration (1 mg/ml) of both synergists was used for Lab-PK. For the control acetone was used alone. Bioassays were performed according to method described above.

2.6. Data analysis of bioassays

The toxicological data was analyzed with POLO software (LeOra Software, 2005) by probit analysis (Finney, 1971) to determine the LC_{50} (median lethal concentration) values, their standard errors, slopes, and confidence intervals (CIs).

Synergism ratio (SR) for synergism bioassays was evaluated as follows:

SR = LC_{50} of insect population exposed to insecticide/LC_{50} of population exposed to insecticide with synergist.

2.7. Realized heritability estimation

Realized heritability (h^2) was estimated by using the method of Falconer et al. (1996) and Tabashnik (1992) by the following equation.

h^2 = Selection response/Selection differential

Selection response was calculated as:

Selection response = (Log final LC_{50} − Log Initial LC_{50})/N