Crop Protection 94 (2017) 38–43

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Resistance risk assessment to chlorpyrifos and cross-resistance to other insecticides in a field strain of *Phenacoccus solenopsis* Tinsley

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ARTICLE INFO

Article history:
Received 22 August 2016
Received in revised form
30 November 2016
Accepted 7 December 2016

Keywords:
Cotton
Mealybug
Invasive pest
Organophosphate
Resistance evolution

ABSTRACT

The organophosphate insecticide chlorpyrifos is recommended for control of a number of insect pests, including cotton mealybug, *Phenacoccus solenopsis* Tinsley (Homoptera: Pseudococcidae) in Pakistan. This work assessed chlorpyrifos resistance evolution and cross-resistance to other insecticides. After 23 generations of selection, the *P. solenopsis* strain (Chlor-SEL) had a 26652-fold level of resistance to chlorpyrifos compared to a susceptible strain. Realized heritability (h^2) of resistance to chlorpyrifos was 0.04. The Chlor-SEL strain also had a low level of cross resistance to lambda-cyhalothrin (14-fold) and a very low level cross-resistance to nitenpyram and profenofos after 23 generations of selection. The projected rate of resistance development indicated that if 50–90 percent of a *P. solenopsis* population were selected with chlorpyrifos, a ten-fold increase in the lethal concentration 50 (LC_{50}) would occur in 22–10 generations ($h^2 = 0.04$, Slope = 0.70). At a similar slope, if $h^2 = 0.14$, then only 6-3 generations are required for a ten-fold increase in the LC_{50} at 50–90 percent selection intensity, respectively. Likewise, if $h^2 = 0.24$, then the same would occur in 4–2 generations. This study showed that *P. solenopsis* has the ability to become resistant to chlorpyrifos but insect resistance management strategies such as rotation of different group of insecticides are needed to prolong the effectiveness of chlorpyrifos in controlling *P. solenopsis*.

1. Introduction

Cotton mealybug, *Phenacoccus solenopsis* Tinsley (Homoptera: Pseudococcidae), is a major insect pest of cotton, vegetables, ornamental and medicinal plants worldwide (Abbas et al., 2010; Afzal et al., 2015d; Fand and Suroshe, 2015; Wang et al., 2009) due to its polyphagous nature. In Pakistan, *P. solenopsis* caused havoc to cotton production in 11 growing districts of Punjab during 2005 (Saeed et al., 2007) and average cotton yield was reduced by 50% (Muhammad, 2007). Apart from Pakistan, *P. solenopsis* has also created economic losses for cotton growers in India (Nagrare et al., 2009), United States of America (Fuchs et al., 1991), Republic of China (Wang et al., 2009), Taiwan and Thailand (Hodgson et al., 2008), Australia (Charleston et al., 2010) and Turkey (Kaydan et al., 2013). *P. solenopsis* damage plants by sucking cell sap from the phloem and secreting honey dew that results in the development of a sooty mold which affects the photosynthesis process and can cause the premature death of plants (Afzal et al., 2015a; Culik and Gullan, 2005; Wang et al., 2010b).

Synthetic chemical insecticides are used for the management of *P. solenopsis* worldwide, including Pakistan. However, the unnecessary and over use of insecticides in cotton agroecosystem has led to the development of resistance by *P. solenopsis* (Saddiq et al., 2014, 2015). Resistance in *P. solenopsis* under laboratory conditions has previously been documented to insecticides including acetamiprid (Afzal et al., 2015a,d), chlorpyrifos (Afzal et al., 2015b), emamectin benzoate (Afzal and Shad, 2015), deltamethrin (Saddiq et al., 2016), and indoxacarb (Afzal et al., 2015e). Extensive use of the organophosphate chlorpyrifos has resulted in resistance reported in a range of pests including *P. solenopsis*, *Tetranychus urticae* (Koch), *Laodelphax striatellus* (Fallén), and *Liriomyza sativae* (Blanchard) (Afzal et al., 2015b; Askari-Saryazdi et al., 2015; Kumral et al., 2009; Recep and Yorulmaz, 2010; Saddiq et al., 2016; Wang et al., 2010a).

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http://dx.doi.org/10.1016/j.cropro.2016.12.011
0261-2194/

Frequent use of different insecticides may result in loss of efficacy due to cross-resistance in the insect populations which imposes difficulties in developing successful insecticide resistance management plans (Kranthi et al., 2001; Basit et al., 2011). Cross-resistance to other insecticides due to selection of chlorpyrifos resistance has been reported in *L. striatellus* (Wang et al., 2010a), *L. sativae* (Askari-Saryazdi et al., 2015), and *Sogatella furcifera* (Horváth) (Mu et al., 2016). Studying resistance and cross-resistance is useful to limit the development of resistance by employing practices such as insecticide mixtures and rotation of insecticides with different modes of action (Shen and Wu, 1995; Abbas et al., 2015).

Risk assessment of insecticide resistance using laboratory or field selection studies can help to avoid or postpone resistance problems in the field (Jutsum et al., 1998; Lai and Su, 2011). Laboratory insecticide selection provides a quick way with fewer variables than those that occur in the field and can reveal the maximum potential of an insect to become resistant (Abbas and Shad, 2015; Sial and Brunner, 2010; Lin et al., 2003; Tabashnik, 1992). In this work, we studied the impact of continuous selection with chlorpyrifos on resistance allele frequencies (h^2) under laboratory conditions in *P. solenopsis* and observed cross-resistance to other insecticides such as profenofos, lambda-cyhalothrin and nitenpyram in the Chlor-SEL strain. The results of this study will be helpful in our understanding of chlorpyrifos resistance and its management in controlling *P. solenopsis*.

2. Materials and methods

2.1. Insects

Approximately 300 insects (nymphs and adults) were randomly selected from ten different areas of a cotton field located in Multan (30.1978° N, 71.4697° E). At the time of collection, the cotton plants were at the reproductive stage. The cotton field of the collection site received heavy amount of sprays from organophosphates, pyrethroids and new chemicals classes during the growing season to control various sucking and chewing pests prior to collecting insects (Afzal et al., 2015b,d; Saddiq et al., 2014, 2015). After collection, the insects were transported in plastic jars (12 × 24 cm) to the laboratory and were maintained at 27 ± 2 °C, 65 ± 5% R.H. and 16:8 h L:D and reared on China rose, *Hibiscus rosasinensis* L. leaves and tender shoots. All stages of *P. solenopsis* were kept in plastic jars (12 × 24 cm) covered with a muslin cloth. The culture was refreshed every 2–3 days with clean fresh leaves along with small twigs. For a reference susceptible strain, a field strain was collected from a cotton field located in Multan district and reared without insecticide exposure for more than one year in the laboratory (Afzal et al., 2015b).

2.2. Insecticides

Commercial formulations of chlorpyrifos (Lorsban®, 40EC; Dow Agro Sciences, Pakistan), lambda-cyhalothrin (Karate® 2.5EC, Syngenta), profenofos (Curacron® 500EC, Syngenta) and nitenpyram (Paranol® 10EC, Kanzo Agro Chemicals) were used for the experiments.

2.3. Bioassays

To assess the toxicities of selected insecticides, a leaf dip bioassay was conducted on 2nd instar nymphs of *P. solenopsis* (Afzal et al., 2015d). Serial dilutions of insecticide concentrations (μg a.i/ml) were prepared using chlorpyrifos, lambda-cyhalothrin, profenofos, and nitenpyram. Five concentrations were used for each bioassay and each concentration was replicated five times. The five concentrations ranged between 0.625 and 10 μg a.i/ml for the susceptible, 31.25–1000 μg a.i/ml for the field population (Field Pop; G3), and 62.5–8000 μg a.i/ml for the chlorpyrifos selected strain (Chlor-SEL; G5-G25). Fresh leaves were dipped into serial dilutions of insecticides for 10 s and air dried at room temperature. Leaves for control were immersed into water only. Treated dried leaves were placed into petri-dishes (5 cm diameter). Five 2nd instars nymphs were placed in each petri dish so a total of 150 nymphs were used for a single bioassay (including the control). Bioassays were kept under the laboratory conditions as mentioned above. Mortality data were assessed 48 h after exposure to chlorpyrifos, lambda-cyhalothrin and profenofos, and 72 h after exposure to nitenpyram. Nymphs were considered to be dead if there was no leg movement after a gentle touch with fine hairbrush (Afzal et al., 2015d).

2.4. Chlorpyrifos selection

The field population was selected with chlorpyrifos for 23 generations (G_3-G_{25}) and designated as the Chlor-SEL strain. Selection was carried out with the leaf dip method by exposing 2nd instar nymphs of *P. solenopsis* to chlorpyrifos (Afzal et al., 2015b). Selection was done at each generation and averages of 300 nymphs were exposed to increasing concentrations (75.26–2601.42 μg a.i/ml). The selection of chlorpyrifos concentrations was based on the objective of having a sufficient number of nymphs to produce the next generation. Nymphal mortality was assessed after 48 h exposure to chlorpyrifos and the survivors of each selection were reared to obtain the next generation.

2.5. Estimation of realized heritability

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

$$h^2 = \frac{\text{Selection Response(R)}}{\text{Selection differential}(S)}$$

We estimated R, the difference in mean phenotype and whole parental generation before selection by Falconer (1989):

$$\text{Selection response(R)} = \frac{\text{Log final LC}_{50} - \text{Log initial LC}_{50}}{\text{N}}$$

The final LC_{50} was the LC_{50} value after N number of generations and the initial LC_{50} was the LC_{50} value of the field population before selection. Selection differential was calculated as:

$$\text{Selection differential(S)} = \text{i} \times \sigma\text{p}$$

Intensity of selection was calculated as follows:

$$\text{i} = 1.583 - 0.0193336\text{p} + 0:0000428\text{p}^2 + 3.65194/\text{p}$$

where p is the average percent survival of Chlor-SEL strain after N number of selection (Tabashnik and McGaughey, 1994) and σp is the phenotypic standard deviation calculated by:

$$\sigma p = [(\text{initial slope} - \text{final slope})0.5]^{-1}$$

To estimate changes in R, S, and h^2 during the selection pressure, each parameter was determined for the first and second half (7 generations in one half) of the experiment. The generation G13 was used in both halves of the experiment. The generation (G) needed for a ten-fold increase in LC_{50} was calculated by: