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Resistance in the mealybug *Phenacoccus solenopsis* Tinsley (Homoptera: Pseudococcidae) in Pakistan to selected organophosphate and pyrethroid insecticides

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ABSTRACT

The mealybug *Phenacoccus solenopsis* is a destructive pest of cotton with the potential to develop resistance to most chemical classes of insecticides. Six populations of *P. solenopsis* from cotton crops at six different locations in Pakistan were evaluated for resistance to selected organophosphate and pyrethroid insecticides. Resistance ratios (RRs) at LC_{50} were in the range of 2.7–13.3 fold for chlorpyrifos, 11.6–30.2 fold for profenofos and for the three pyrethroids tested were 10.6–46.4 for bifenthrin, 5.8–25.2 for deltamethrin and 4.1–25.0 for lambda-cyhalothrin. This is the first report of resistance to organophosphate and pyrethroid insecticides in Pakistani populations of *P. solenopsis*. Regular insecticide resistance monitoring programs are needed to prevent field control failures. Moreover, integrated approaches including the judicious use of insecticides and rotation of insecticides with different modes of action are needed to delay the development of insecticide resistance in *P. solenopsis*.

1. Introduction

The cotton mealybug, *Phenacoccus solenopsis* Tinsley (Homoptera: Sternorrhyncha: Coccoidea: Pseudococcidae), has a worldwide distribution with its reported origin in Central America (Williams and Granara de Willink, 1992). *P. solenopsis* has a wide host range and causes economic damage to several crops including ornamentals, medicinal plants, vegetables and cotton (Arif et al., 2009). Since 2005, *P. solenopsis* has been causing severe damage to the cotton crop in Pakistan (Hodgson et al., 2008), mainly by sucking plant sap and producing sugary material on which sooty mold develops, which ultimately blocks photosynthesis (Saeed et al., 2007). In Pakistan, the incidence of *P. solenopsis* was first observed on cultivated cotton at Vehari Agricultural Farm during the cotton season 2005. The pest has now spread throughout the cotton growing areas of the country and it caused an epidemic in Vehari, Multan and Bahawalpur districts of Punjab during 2006. In the Sindh province of Pakistan, the situation was even worse and severe infestation was observed first time on an area of about 3000 acres in 2005 and 2006 (Sahito et al., 2011). Attacked plants remain stunted and produce fewer bolls; leaves turn yellow, dry up and eventually fall off (Dhawan et al., 1980; Mark and Gullan, 2005). Pakistan is the third largest exporter of cotton in the world, so there is a need for effective management of different pests of major economic importance to the farming community of Pakistan (Hodgson et al., 2008). There is no defined integrated pest management plan for *P. solenopsis* which leads to the haphazard and inappropriate use of synthetic insecticides including over or under dosing of insecticide applications. For example, farmers started to use similar insecticides for control of *P. solenopsis* which they usually use for control of other cotton insect pests. Presently, insecticides from different insecticide classes including organophosphates, carbamates, pyrethroids (Saeed et al., 2007) and new chemicals (David et al., 2010) are being used for controlling *P. solenopsis* and other coexisting pest species [e.g., *Aphis gossypii*

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(Glover), *Bemisia tabaci* (Gennadius), *Thrips tabaci* (Linnaeus), *Helicoverpa armigera* (Hubner), *Spodoptera litura* (Fabricius)] in cotton growing areas of Pakistan (Lysandrou et al., 2012). Over reliance on insecticides, however, could result in the environmental pollution and development of resistance which ultimately causes the control failures in the field.

The development of insecticide resistance in an area is a result of inappropriate use of insecticides (Shad et al., 2012). In the Indo-Pakistan subcontinent, there is extensive use of the broad spectrum conventional insecticides such as organophosphates and pyrethroids which provides strong selective pressure for the development of insecticide resistance in different pests (Ahmad et al., 2007; Kranthi et al., 2002). Increased application rates of insecticides at short intervals between applications, result in resistance development. Previously, insecticide resistance has been reported in *H. armigera* (Ahmad et al., 1995), *Amrasca devastans* (Dist.) (Ahmad et al., 1999), *A. gossypii* (Ahmad et al., 2003), *S. litura* (Shad et al., 2012), *Spodoptera exigua* (Ishtiaq and Saleem, 2011), *Aedes albopictus* (Skuse) (Khan et al., 2011), *Musca domestica* (Linnaeus) (Khan et al., 2013a,b) and *B. tabaci* (Basit et al., 2011), against different insecticides from Pakistan. Recent reports of control failure of *P. solenopsis* in cotton growing areas in Punjab, Pakistan (Anonymous, 2011) revealed that insecticide resistance could be the probable reason for such failures in the field. However, to the best of author's knowledge, this is the first report of resistance to conventional insecticide in *P. solenopsis* from Pakistan. In the present work, we performed experiments to assess resistance to the selected organophosphate and pyrethroid insecticides from Punjab, Pakistan, and to define baseline data for future monitoring efforts.

2. Materials and methods

2.1. Insects

Second nymphal instars of *P. solenopsis* were collected from the cotton plants selected randomly from six different locations (Fig. 1): Multan (30.1978° N, 71.4697° E), Khanewal (30.3030° N, 71.9309° E), Muzaffargarh (30.0703° N, 71.1933° E), Mailsi (29.8003° N, 72.1758° E) Bahawalpur (29.3956° N, 71.6836° E) and Sahiwal (30.6644° N, 73.1083° E), of southern Punjab, Pakistan, from the cotton crop during 2011–2013. Each collected sample consisted of varying number of insects. The localities were selected on the basis of heavy insecticide use on the cotton crop for the management of different insect pests including *P. solenopsis*. Populations from the respective localities were reared separately in plastic jars (22 cm × 13 cm) having mesh cloth on upper side for aeration. In order to remove maternal effects and to gain homogeneous population, insects were reared for one generation in laboratory. The insects were fed on China rose, *Hibiscus rosa-sinensis* L., leaves and the culture was reared in the laboratory at 27 ± 2 °C and 60–65% relative humidity with a 12:12 h light:dark photoperiod. China rose leaves were changed after every two days. A laboratory susceptible reference population (Lab PK) was collected from cotton field of Central Cotton Research Institute, Multan in 2010 and maintained for 16 generations without exposure to insecticides. Although not truly susceptible, the population had quite low median lethal concentration (LC_{50}) values compared to the rest of the populations and hence could serve as a baseline for monitoring resistance in the future (Khan et al., 2013a).

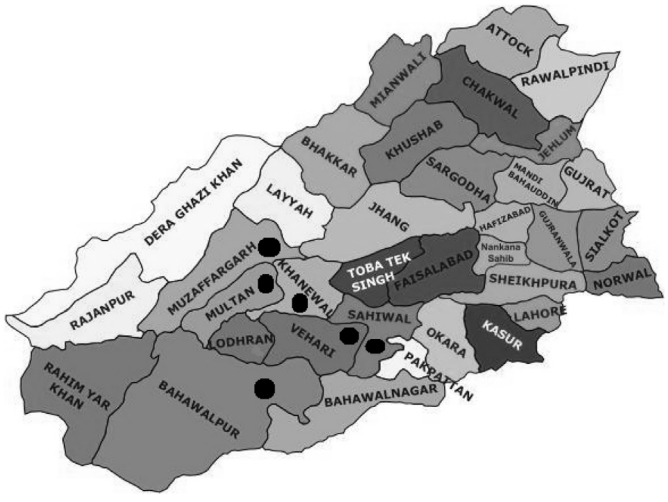

Fig. 1. Sampling sites of *Phenacoccus solenopsis* in various zones of Punjab province of Pakistan.

2.2. Insecticides

The insecticides used for bioassays were: chlorpyrifos (Lorsban, 400 EC; Dow Agro Sciences, Pakistan Limited), lambda cyhalothrin (Karate, 2.5 EC; Syngenta, Pakistan Limited), profenofos (Curacron, 500 EC; Syngenta, Pakistan Limited), bifenthrin (Talstar, 10 EC; FMC, Philadelphia, PA) and deltamethrin (Decis super, 10 EC; Bayer Crop Sciences, Pakistan Limited).

2.3. Bioassays

Insecticidal bioassays with all the populations were conducted at G_1 on the second nymphal instar of *P. solenopsis* by using a leaf dip method (Bielza et al., 2008). Briefly, five concentrations (causing >0 and <100% mortality) of each insecticide were made in tap water. The contractions were made by making a stock solution and then serial dilutions were made. Fresh China rose leaves were dipped in each concentration for 10 s and air-dried at room temperature for 1–1.5 h. After drying, the treated leaves were kept in Petri dishes (5 cm in diameter). Moist filter paper was used in Petri dishes to prevent the desiccation (Afzal et al., 2014). The large sized leaves were used to fully cover the Petri dishes. There was no insect loss because the Petri dishes were closed tightly. Five Petri dishes were prepared for each concentration. Five second instar nymphs were introduced in each Petri dish, and each treatment (concentration) was replicated 5 times, together with control. For the control, China rose leaves dipped in tap water (without insecticides) were presented to the nymphal instars. Mortality was checked after 48 h exposure to insecticides and nymphs were considered dead if they failed to move after a gentle touch with a needle (Saeed et al., 2007). Bioassays were done at 27 ± 2 °C and 60–65% relative humidity with a 12:12 h light:dark photoperiod.

2.4. Data analysis

The mortality data, if needed, was corrected by Abbott's formula (Abbott, 1925) and analysed by probit analysis (Finney, 1971) with EPA probit analysis program (version 1.5) (EPA, 1999). The control mortality, in almost all the cases, was below 10%. The median lethal concentrations (LC) were determined and any two values were considered significantly different if their respective 95% confidence limits (CL) did not overlap. Resistance ratios (RR) were calculated at LC_{50} by dividing LC_{50} values of each insecticide by the corresponding LC_{50} value for the Lab PK population. LC_{50} values were compared to those obtained with the Lab PK population and the resulting levels of insecticide resistance were scaled based on following the criterion: resistance ratio (RR) = 1, indicates no