Monitoring resistance of *Phenacoccus solenopsis* Tinsley (Homoptera: Pseudococcidae) to new chemical insecticides in Punjab, Pakistan

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**Article info**

**Abstract**

Mealybug, *Phenacoccus solenopsis* Tinsley is a devastating pest of cotton and many other crops and ornamental plants. *P. solenopsis* has ability to develop resistance to most chemical classes of insecticides. The aim of present study was to monitor the resistance to new chemical insecticides in different field populations of *P. solenopsis*. Six populations of *P. solenopsis*, collected from Multan, Khanewal, Muzaffar Garh, Mailsi, Bahawalpur, and Sahiwal, were tested for resistance to selected new chemical insecticides by the leaf dip method. The resistance ratios were in the range of 4.0–30.9-fold for nitrophen, 12.6–105.0-fold for acetamiprid, 3.4–79.0-fold for emamectin benzoate, 0.4–2.3 fold for indoxacarb, 12.1–28.9 for pyriproxyfen, 13.0–37.4 for cyromazine, 7.2–35.0 for methoxyfenozide, and 5.5–170.0-fold for lufenuron compared to the laboratory susceptible strain. Regular insecticide resistance monitoring and integrated management plans including the judicious use of insecticides with correct application rates and methods and rotation of insecticides with different modes of action are required to delay insecticide resistance development in *P. solenopsis*.

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1. Introduction

The mealybug, *Phenacoccus solenopsis* Tinsley (Homoptera: Pseudococcidae) is a devastating pest of cotton in different regions of the world such as Pakistan (Afzal et al., 2015; Hodgson et al., 2008), India (Nagrare et al., 2009), China (YanPing et al., 2009), United State (Kumashiro et al., 2001), Taiwan and Thailand (Hodgson et al., 2008), due to its polyphagous nature. This pest has a wide host range and causes economic damage to several crops including cotton, ornamentals, medicinal plants and vegetables (Abbas et al., 2010; Nagrare et al., 2009; YanPing et al., 2009). *P. solenopsis* causes severe damage to cotton (Abbas et al., 2009; Wang et al., 2010), mainly by sucking plant sap and producing sugary material on which sooty mold develops, which ultimately blocks photosynthesis (Saeed et al., 2007). Attacked plants remain stunted and produce fewer bolls, leaves turn yellow, dry up and eventually fall off (Culik and Gullan, 2005).

Insecticides from different classes are being used for the control of different cotton pests such as *Bemisia tabaci* Gennadius, *Thrips tabaci* L., *Helicoverpa armigera* Hübner, *Spodoptera litura* Fabricius (Ahmad et al., 2007, 2008; Lysandrou et al., 2012) and *P. solenopsis* (Afzal et al., 2015) in cotton growing areas of Pakistan. There is no defined integrated pest management plan which leads to the hazardous use of new insecticidal classes. The development of insecticide resistance in an area generally depends upon the extensive use of insecticides (Shad et al., 2012). Resistance to new chemical insecticides (neonicotinoids, spinosys, avermectins, oxadiazine, insect growth regulators) has been reported in different insect pests such as in *Aedes albopictus* Skuse (Khan et al., 2011), *Aphis gossypii* Glover (Nauen and Denholm, 2005), *B. tabaci* Basit et al. (2008), *B. armigera* (Faheem et al., 2013), *Musca domestica* L. (Abbas et al., 2014; Kaufman et al., 2010; Khan et al., 2013b), *Myzus persicae* Sulzer (Srigiriraju et al., 2010), *Nilaparvata lugens* Stål (Zhang et al., 2014), *S. litura* (Ahmad et al., 2008; Shad et al., 2012) and *Spodoptera exigua* Hübner (Ishfaq et al., 2012). The development of resistance is promoted by a number of biological traits such as adaptability to different environments, high fecundity, short developmental time, and cross-

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resistance.

In areas of intensive insecticide use, such as cotton growing areas of Pakistan, monitoring the development of insecticide resistance in insect pests is of the utmost important in order to select appropriate insecticides, and to retain their efficacy for a long time (Khan et al., 2013b, Saddiq et al. (2014) reported resistance in P. solenopsis to organophosphate and pyrethroid insecticides. In the present work, we were interested to assess resistance to the selected new chemical insecticides from Punjab, Pakistan in P. solenopsis, and to provide baseline data for future monitoring efforts that will help to define a control strategy for the management of P. solenopsis resistance.

2. Materials and methods

2.1. Insects

Populations of P. solenopsis were collected from cotton plants selected randomly from six different locations: Multan (30.1978°N, 71.4697°E), Khanewal (30.3030°N, 71.9309°E), Muzaffar Garh (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) in Punjab, Pakistan, during 2011–2013. The selection of localities was based on heavy insecticide use by farmers on cotton for the management of different insect pests (Ahmad et al., 2008) including P. solenopsis. Populations from the respective localities were reared separately for one generation under laboratory conditions in plastic jars (22 × 13 cm) covered with mesh cloth on upper side for aeration, in order to remove maternal effects and to gain a homogeneous population. The insects were fed on China rose (Hibiscus rosasinensis L.) leaves, and the culture was maintained in the laboratory at 27 ± 2 °C and 60–65% relative humidity with a 14:8 h (light:dark) photoperiod. China rose leaves were changed twice a week (Saddiq et al., 2014). The population collected from fields of Central Cotton Research Institute, Multan in 2010 was maintained for 16 generations in the laboratory without exposure to insecticides to restore the insecticide susceptible genes. This population was designated as Lab Pk and was used as insecticide susceptible reference population. Although not truly susceptible, the population had quite low median lethal concentration (LC50) from Multan and Sahiwal showed tolerance to this insecticide. The populations collected from Multan and Sahiwal showed very high resistance level (105.0-fold) and Khanewal and Mailsi populations showed high level of resistance (59.7- to 95.3-fold).

2.2. Insecticides

The commercial formulated insecticides used for bioassays were: nitenpyram (Paranol, 10EC; Kanzo, Agrochemicals), acetamiprid (Mospilan, 20SP; Arysta Life Sciences), Emamectin benzoate (Proclaim, 1.9EC; Syngenta), Indoxacarb (Steward, 150EC; Arysta Life Sciences), pyriproxyfen (Admiral, 10EC; FMC), cyromazine (Trigard, 75WP; Syngenta), methoxyfenozide (Runner, 240SC; Arysta Life Sciences), and lufenuron (Match, 050EC; Syngenta).

2.3. Bioassays

Bioassays on second instar nymphs of P. solenopsis of all populations were carried out by using a leaf-dip method (Saddiq et al., 2014). Five concentrations of each insecticide were prepared as serial dilutions for each insecticide. Fresh China rose leaves were dipped in each concentration for 10 s and kept to dry at room temperature for 1–1.5 h. The treated leaves were kept in Petri dishes and five Petri dishes were prepared for each concentration. Five second instar nymphs were introduced in each Petri dish, and each treatment was repeated 5 times. A total of 25 nymphs were used for each concentration. For the control, China rose leaves dipped in tap water (without insecticides) were presented to the nymphal instars. Mortality was assessed after 72 h exposure to nitenpyram, emamectin benzoate, acetamiprid, and indoxacarb and 96 h exposure to pyriproxyfen, cyromazine, methoxyfenozide, and lufenuron. Nymphs were considered dead if they failed to move after a gentle touch with a fine brush (Afzal et al., 2015).

2.4. Data analysis

The mortality data were corrected by Abbott’s formula (Abbott, 1925), if necessary and analyzed by probit analysis (Finney, 1971) with EPA Probit Analysis Program (version 1.5) (EPA, 1999) to determine LC50 values and their 95% confidence intervals (CI). Resistance ratios (RR) were calculated by dividing the LC50 value of test strains by the LC50 of the Lab Pk. The 95% CI of RR were calculated according to Robertson and Preisler (1992) and considered significant if these did not include the value of 1. An alpha level of 0.05 was used for all comparisons.

Resulting RR values of each insecticide were scaled based on the following criterion: resistance ratio (RR) = 1 indicates no resistance; RR = 2–10, tolerance; RR = 11–20, low resistance; RR = 21–50, moderate resistance; RR = 51–100, high resistance, and RR > 100, very high resistance (Ahmad and Arif, 2009; Torres-Vila et al., 2002).

3. Results

3.1. Neonicotinoids

Results of the concentration–response bioassays showed tolerance to moderate resistance levels for nitenpyram tested P. solenopsis populations compared with the Lab Pk (Table 1). A moderate level of resistance was found in populations collected from Khanewal (30.9-fold), and Mailsi (29.2-fold). Low resistance levels (14.6- to 16.2-fold) were observed in Muzaffar Garh and Bahawalpur populations of P. solenopsis. The populations collected from Multan and Sahiwal showed tolerance to this insecticide.

Low to very high resistance levels to acetamiprid were observed in tested P. solenopsis populations (Table 1). Populations collected from Multan, Bahawalpur and Sahiwal showed low resistance levels (12.6- to 18.5-fold), Muzaffar Garh population showed very high resistance level (105.0-fold) and Khanewal and Mailsi populations showed high level of resistance (59.7- to 95.3-fold).

3.2. Avermectins and oxadiazine

No to high resistance levels for emamectin benzoate were observed in tested P. solenopsis populations compared with the Lab Pk (Table 1). A high level of resistance (79.1-fold) was found in population collected from Muzaffar Garh. No resistance was observed in Sahiwal population Other P. solenopsis populations showed low level of resistance (10.3- to 18.2-fold). All the tested populations of P. solenopsis had no resistance to indoxacarb compared with the Lab Pk except Khanewal and Muzaffar Garh which had tolerance to this insecticide (Table 1).

3.3. Insect growth regulators

Low to moderate resistance levels for pyriproxyfen were observed in tested P. solenopsis populations compared with the Lab Pk (Table 1). Moderate level of resistance was found in populations collected from Muzaffar Garh (23.5-fold), and Mailsi (28.9-fold). Other P. solenopsis populations showed low resistance levels (12.1- to 17.1-fold).

Low to very high resistance levels for cyromazine were observed
in tested *P. solenopsis* populations (Table 1). The populations of *P. solenopsis* collected from Multan, Muzaffar Garh, and Sahiwal showed low resistance levels (13.0- to 17.2-fold). *P. solenopsis* populations collected from Khanewal, Mailsi, and Bahawalpur showed moderate resistance levels (26.1- to 37.4-fold).

Of the 6 populations of *P. solenopsis* tested, populations collected from Multan, and Sahiwal showed tolerance to methoxyfenozide (Table 1). A moderate level of resistance (35.0-fold) was observed in population collected from Mailsi. Other *P. solenopsis* populations collected from Khanewal, Muzaffar Garh, and Bahawalpur showed low resistance levels (15.7- to 19.8-fold). For lufenuron, all the tested populations of *P. solenopsis* showed low level of resistance except Sahiwal which had tolerance to this insecticide (Table 1).

### 4. Discussion

The results of present study suggest the prevalence of varying levels of insecticide tolerance in *P. solenopsis* compared with the Lab PK. However, insects should not be assumed resistant until ten-fold of resistance is observed (Khan et al., 2013b; Valles et al., 1997). Our results revealed less than ten-fold resistance ratios in *P. solenopsis* from two locations to nitenpyram, one location to emamectin benzoate, all locations to indoxacarb, two locations to methoxyfenozide and one location to lufenuron due to tolerance rather than resistance (Table 1).
than resistance. There was more than ten-fold resistance in all other populations studied from different locations for all tested insecticides. Previously, varying resistance to novel chemistry insecticides had been reported in different insect pests from Pakistan like *S. litura* (Ahmad et al., 2008; Shad et al., 2012), *B. tabaci* (Basit et al., 2013), *A. albopictus* (Khan et al., 2011) and *M. domestica* (Abbas et al., 2015; Khan et al., 2013b). However, the occurrence of resistance to new chemical insecticides in *P. solenopsis* from Pakistan is reported here for the first time. The mixing of new chemicals (neonicotinoids, spinosyns, avermectins, oxadiazine, insect growth regulators) with organophosphate and pyrethroids could also be responsible for multiple resistance problems. In Pakistan, it is a common practice to mix organophosphates, pyrethroids and new chemicals (neonicotinoids, spinosyns, avermectins, oxadiazine, insect growth regulators) to control various cotton pests which could lead cross/multiple resistance between these compounds.

In the present study, none to moderate levels of resistance to nitenpyram and low to very high levels of resistance to acetamiprid were observed in *P. solenopsis* collected from six locations. Resistance to these insecticides had previously been reported in different insect pests worldwide such as *B. tabaci* (Basit et al., 2011; Hounde et al., 2010), *Plutella xylostella* (Ahmad et al., 2008; Shono et al., 2004) and *M. domestica* (Kaufman et al., 2010). In Pakistan, nitenpyram and acetamiprid are among the most efficient neonicotinoid insecticides for the control of sucking insect pests such as whiteflies, aphids, thrips, cotton mealybug, leaf- and plant hoppers, and a number of coleopteran pests (Basit et al., 2011). The most probable reason for the high resistance levels to neonicotinoids is due to heavy use of these insecticides for the control of cotton sucking pests in Pakistan.

In present results, no resistance levels to indoxacarb were observed in all the tested populations of *P. solenopsis*. Previously, resistance to indoxacarb had been reported in many insect pests worldwide such as *P. xylostella* (Sayyed et al., 2008; Sayyed and Wright, 2006), *S. litura* (Ahmad et al., 2008) and *M. domestica* (Shono et al., 2004). Despite the heavy usage of indoxacarb on cotton for the management of many pests in Pakistan, this insecticide is still effective for the control of *P. solenopsis*. Therefore, the reason for no resistance in indoxacarb might be due to an independent mechanism of indoxacarb resistance or low usage of this insecticide at these locations.

The present results showed no to high resistance levels for emamectin benzoate. Resistance to emamectin benzoate had been reported in many pests (Khan et al., 2011; Liang et al., 2003; Pu et al., 2010; Shad et al., 2010; Zaka et al., 2014). Emamectin benzoate is used for the control of lepidopteran pests (*Ishaaya et al., 2002*), which can also select other non target organisms (Abbas et al., 2012) resulting in resistance. The high resistance to emamectin benzoate might be due to cross-resistance to other insecticides which were being used for the management of cotton pests including *P. solenopsis*.

The present results indicated that very low to moderate resistance levels to insect growth regulators were observed in tested populations of *P. solenopsis*. Previously, resistance to insect growth regulators have been reported in a variety of insect pests, e.g. *B. tabaci* (Crowder et al., 2008; Ma et al., 2010), *M. domestica* (Bell et al., 2010; Tang et al., 2002), *P. xylostella* (Cao and Han, 2013; Sun et al., 2012) and *S. litura* (Ahmad et al., 2008; Rehan and Freedé, 2010; Shad et al., 2010). The variability in resistance could be due to different selection pressures on each population and different resistance mechanisms among the populations resulting in either a delay or an increased rate of resistance development (Silva et al., 2011). Heavy usage of insecticides could be the most probable reason for the development of insecticide resistance in *P. solenopsis* in Punjab, Pakistan, because there is no defined resistance management plans for *P. solenopsis*. Since cotton is considered as the backbone of Pakistan’s agricultural economy (Saeed et al., 2007), cotton growers make special efforts to maximize its yield. Insect pests are amongst major factors in reducing the yield of cotton in Pakistan and presently more than 34 different pesticides have been used to manage different insect pests (Saleem et al., 2008). *P. solenopsis* has caused major economic damage to cotton in Pakistan since 2005. The growers’ response was to use a tremendous amount of insecticides to manage the pest. Consequently, for example, in 2007, insecticides of US$ 121.4 million worth were used on cotton alone in only two months in Punjab (Hodgson et al., 2008). Moreover, previous studies (Khan et al., 2013b) revealed that the decision making by the farming community regarding the dose of pesticides and modes of action to manage insect pests largely depended on the availability, the amount of chemicals, and application equipment. Injudicious use with improper application techniques could also be responsible for the development of resistance to various insecticides (Khan et al., 2013a,b; Silva et al., 2011).

The evolution of insecticide resistance depends upon the selection pressure caused by inappropriate chemistry, which ultimately increases the number of resistant individuals in the population. Despite heavy insecticide usage on cotton, none to low levels of resistance to indoxacarb, emamectin benzoate and insect growth regulators is interesting. Different agro-ecological factors such as presence of refugia that harbor less resistant or susceptible individuals could dilute resistant gene frequencies. In addition, fitness cost might be associated with resistant genes, allowing the insects to remain at an acceptable level (Khan et al., 2014; Sayyed et al., 2005). However, further studies are needed to confirm the exact phenomenon by selecting *P. solenopsis* with different insecticides.

The results of present work revealed that the occurrence of insecticide resistance in *P. solenopsis* might be the result of a lack of systematic management practices (Sayyed et al., 2005). It is necessary to develop efficient management plans as soon as possible for delaying further resistance development and the failure of products. Resistance to different insecticides with different modes of action revealed that the phenomenon of cross-resistance or multiple-resistance may also be present in the tested populations of *P. solenopsis*. However, further studies are needed to confirm these findings and to design effective management plans. Developing countries, such as Pakistan, have issues with insecticide resistance due to the indiscriminate use of insecticides for control of different insect pests of agricultural or medical importance (Abbas et al., 2015; Sayyed et al., 2005). There are many problems including the use of inappropriate pesticides with incorrect dosage, the use of low quality pesticide formulations, and the use of inappropriate application methods. The appropriate use of insecticides may decrease the severity of resistance and control failures of *P. solenopsis* management on cotton in Punjab, Pakistan. Restricted use of insecticide to which resistance has developed, insecticide mixtures, and rotation of insecticides with unrelated modes of action could be helpful for resistance management in *P. solenopsis* (Kaufman et al., 2001; Memmi, 2010). In addition, standard resistance monitoring activities should be planned that would help to identify the effectiveness of insecticides for *P. solenopsis* management. Insecticides with novel modes of action like insect growth regulators are more compatible with integrated pest management (IPM) programs as compared to the older insecticides (Saleem et al., 2008). IPM also includes the use of cultural practices, economic thresholds, and preservation of natural enemies. High feeding potential of *Chrysoperla carnea* (Stephens) and *Crypsolaemus
montrouzieri (Mulsant), have been observed against nymphal stages of *P. solenopsis* (Khan et al., 2012) which suggested the use of these bio-control agents as an alternate tools in IPM. Moreover, pre-and post-planting cultural practices such as removal of alternate hosts could help to delay the onset of *P. solenopsis* during the cropping season, thus reducing insecticide usage and selection pressure.

**Conflicts of interest**

The authors declare that they have no conflict of interest.

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**References**


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